JOURNAL OF AGRICULTURAL RESEARCH

VOL. 37 WASHINGTON, D. C., SEPTEMBER 1, 1928 No. 5

THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION [1]

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INTRODUCTION

From the beginning of the energy-metabolism studies at this college, in 1901–2, under the direction of Armsby, a continuing problem, of obvious significance, has been the effect of the plane of nutrition on the several factors of energy loss and expense in the utilization of food, which, collectively, subtracted from the gross energy of the food, leave the net energy available to the animal for purposes of maintenance and production.

The evolution of Armsby's ideas on this subject has been traced in a recent paper by Forbes, Kriss, and Braman (*11*).[3]

In a number of recent papers from this institute, but especially in the one to which the authors have just referred, evidence has been presented, from metabolism experiments with cattle, for the belief that the heat production—a prominent factor in the determination of net-energy values—is not a rectilinear function of the quantity of the feed. This idea has been expressed in the following language (*11, p. 170*):

> On account of the great variability of computed maintenance values and the fact that the computed maintenance from supermaintenance periods is always a materially lower value than is the directly determined fasting katabolism . . . the writers believe that the heat increment does not thus vary directly as the feed.

The same principle is also implied in the determination of different rates of utilization of feed energy for maintenance, body increase, and milk production, as reported in an earlier paper by Forbes, Fries, Braman, and Kriss (*9*).

The effect of this observation, therefore, has been to throw open the whole problem of the subject of this paper, especially as it has to do with the determination of net-energy values of feeds.

A conception fundamental to the above expressions regarding net-energy values and maintenance requirements is the assumption that the maintenance quota of net energy is the same at all planes of nutrition.

Since the fasting katabolism is the accepted measure of this quota, and since, obviously, this value can be determined only during actual fast, its constancy at all planes of nutrition rests on assumption, or definition.

[1] Received for publication July 18, 1928; issued October, 1928.

[2] The authors take this occasion to express their grateful indebtedness to the members of the Department of Animal Husbandry of this college for many courtesies and for valued cooperation in connection with this research, especially to Prof. F. L. Bentley for the provision of the steers used as subjects, to Dr. J. F. Shigley for veterinary advice and service, and to Asst. Prof. P. T. Ziegler for cooperation in obtaining slaughter data in the study of fast.

[3] Reference is made by number (italic) to "Literature cited," p. 299.

It is impossible, therefore, to separate the maintenance requirement of net energy from the remainder of the heat production (the heat increment) in a critically scientific manner; but it is necessary, nevertheless, that we have some one definite value, conventional if not absolute, for the maintenance requirement of net energy at all planes of nutrition, in order to be able to compute the feed requirements of animals for both maintenance and production, as is necessary for guidance in feeding practice.

The above assumption is typical of a number of such postulates, impossible of proof, and warranted in part by definition, convention, or usefulness, which are necessary to the derivation of any system of values of feeds based upon Armsby's net-energy conception.

Care is necessary, therefore, on the part of the student, not to be confused by the two scientific attitudes expressed in the determination of a net-energy value, based as it is in part on the most refined and particular of animal experimentation, but in part also on arbitrary procedures adopted only as means for the establishment of practical measures and guides.

However great the difficulties and compromises involved in the determination of net-energy values of feeds, the principle of this estimation is exceedingly simple, and certainly correct. The total of the expenses and losses of food utilization, in terms of energy, subtracted from the gross energy of the food, yields the net energy available for maintenance and production; and these expenses and losses are (1) the potential energy of the visible excreta, and of the methane produced by carbohydrate fermentation, and (2) the heat increment—this latter comprising not only all direct expenditures of energy in prehension, mastication, deglutition, fermentation, rumination, peristalsis, digestion, transportation, anabolism, dynamic stimulation, and excretion; but also any indirect increase in heat production, either through voluntary or involuntary activity, *which has resulted from the consumption of feed* (except, in the practice of this institute, that the effects of feed on the activity of the animal may be modified by the computation of the heat production to standard conditions as to time spent in the standing and the lying positions).

The inclusion of the last factor as a part of the heat increment is necessitated by the assumption that the fasting katabolism as determined after a period of maintenance feeding is the measure of the maintenance quota of net energy at all levels of nutrition—because, since the maintenance quota is assumed to be constant, all observed increases of heat production following and due to feed consumption must be considered as a part of the heat increment.

Apparently any possible determinations of net-energy values of feeds must involve certain conventional procedures and general standardization of methods. Such data, therefore, are not absolute, but seem to the writers to be the most significant and useful measures that have been proposed, for purposes of practical guidance, in a complex physiological situation in which perfect order, of the sort implied by these values, does not exist.

PLAN OF EXPERIMENTATION

A series of metabolism experiments was conducted, primarily for the purpose of studying the energy metabolism as related to the plane of nutrition, but also permitting observations on several important associated problems.

TABLE 1.—*Schedule of experimentation, daily rations, and live weights of animals*

Period No.	Steer No.	Preliminary feeding period on experimental rations	Total digestion period	Calorimeter period	Plane of nutrition	Rations fed daily				Average live weight of animals
						Roughage		Concentrate		
						Kind	Amount	Kind	Amount	
							Kgm.		*Kgm.*	*Kgm.*
1	47	Nov. 24-29	Nov. 30-Dec. 17	Dec. 14-17	Twice maintenance	Alfalfa hay	4.29	Corn meal	4.29	486.2
2	36	Dec. 8-13	Dec. 14-31	Dec. 28-31	do	do	4.048	do	4.088	482.9
3	47	Dec. 21-27	Dec. 28-Jan. 14	Jan. 11-14	Half more than maintenance	do	3.250	do	3.250	494.6
4	36	Jan. 1-10	Jan. 11-28	Jan. 25-28	do	do	3.090	do	3.090	490.2
5	47	Jan. 14-24	Jan. 25-Feb. 5	Feb. 2-5	Half less than maintenance	do	1.073	do	1.083	474.8
6	36	Jan. 29-Feb. 6	Feb. 7-19	Feb. 16-19	do	do	1.073	do	1.088	471.2
7	47	Feb. 5-14	Feb. 15-Mar. 4	Mar. 1-4	Maintenance (mixed ration)	do	2.145	do	2.188	484.8
8	36	Feb. 21-28	Mar. 1-18	Mar. 15-18	do	do	2.155	do	2.181	481.2
9	47	Mar. 11-14	Mar. 15-Apr. 1	Mar. 29-31	Maintenance (hay alone)	do	6.50	do		499.0
10	36	Mar. 23-28	Mar. 29-Apr. 15	Apr. 12-15	do	do	6.50	do		490.9
11	47			Apr. 19-23 [a]	Fast					
12	36			May 3-7 [b]	do					

[a] Days 4, 5, and 6 of fast.

[b] Days 5, 6, and 7 of fast.

The schedule of experimentation comprises Table 1. These experiments, 12 in number, consisted of a duplicate series, with two 2-year-old steers, at 5 planes of nutrition—1 at fast, 1 each at 3 other planes of nutrition, and 2 at the remaining plane, which was maintenance.

The rations were composed of corn meal and alfalfa hay, in equal weights of dry matter, at four planes of nutrition other than fast, and a second ration of alfalfa hay alone at the maintenance level.

This outline of experiments, in tabular form, is presented below:

Planes of nutrition studied	Order of treatments in experimental program	Rations fed
Twice the maintenance requirement	I	Corn meal; alfalfa hay, 1 : 1
Half more than the maintenance requirement	II	Corn meal; alfalfa hay, 1 : 1
Maintenance (energy equilibrium)	IV and V {a.	Corn meal; alfalfa hay, 1 : 1
	{b.	Alfalfa hay alone.
Half of the maintenance requirement	III	Corn meal; alfalfa hay, 1 : 1
Fasting	VI	None.

The experiments were of the kind which is standard in the energy metabolism studies with cattle at this institute, a unit ordinarily consisting of a 28-day interval, embracing a 10-day preliminary period on the experimental feeding treatment which is to follow, and an 18-day period during which the visible excreta are quantitatively accounted for, the last three days of the 18 also constituting a continuous respiration-calorimetric period, during which the heat produced and the gaseous metabolism are measured.

The digestion periods were conducted at the times indicated by the second column of dates, and were of the above-mentioned standard 18-day length, except as necessarily altered on account of irregularity of behavior of the steers.

The transition feeding periods, in the course of which the steers were changed from one plane of nutrition to another, were commonly 10 days in length; and the portions of these 10-day intervals during which the steers received exactly the quantities of feed to be given during the digestion periods to follow were as indicated in the first column of dates.

The calorimeter periods, other than those during fast, Nos. 1 to 10, were each, as usual, three days in length, barring one incomplete day in period 9, and each was preceded by a 14-hour preliminary interval during which the calorimeter was got into balance, with the animal inside, and all accessory equipment was established in regulated operation.

The calorimeter periods during fast (Nos. 11 and 12) were four days in length, in addition to the usual 14-hour preliminary period.

The length of this preliminary period was determined in part each by a desire to provide for all ordinary requirements, with a liberal excess as a margin of safety, and by considerations of convenience.

Each calorimeter period began and ended at 6 a. m., and therefore covered 18 hours of the first calendar day, and 6 hours of the last such day, of each experimental interval.

Of each of the two fasting periods (Nos. 11 and 12) the heat production of the last three days only was used as a measure of the maintenance requirement of net energy. These were the fourth,

fifth, and sixth days of fast in the case of steer No. 47, and the fifth, sixth, and seventh days of fast in the case of steer No. 36.

Referring to the above tabular outline of experiments—the quantities of feed given during periods 1 and 2—about twice those required to keep the animals in energy equilibrium—were, for these steers, approximately full feed. They would not regularly consume more without leaving a part.

In periods 3 and 4 the steers received half more than a maintenance ration; in periods 5 and 6, half of the maintenance; in periods 7 and 8, maintenance; and in periods 9 and 10, maintenance again, but with a ration of alfalfa hay alone instead of the mixed ration of grain and hay.

The purpose of the periods on hay alone was to make possible the determination of the net-energy values of the individual feeds used—the alfalfa directly, and the corn by difference.

METHODS OF EXPERIMENTATION AND COMPUTATION

The methods employed in this study were in general the same as those described in the recent published work of this institute, but several new procedures were introduced. These were (1) the adoption of the area of the removed hide as the measure of the surface area of the animal; (2) the use of the respiratory quotient and (3) of the amount of the feed residues in the alimentary tract, as well as the heat production, as usual, as criteria in the standardization of conditions for the determination of the fasting katabolism as the measure of the maintenance quota of net energy, (4) the correction of the heat production to correspond to a uniform live weight and maintenance requirement of net energy, in the comparison of the heat production of an animal at different planes of nutrition, (5) the use of a new method for computing net-energy values of individual feeds for the production of body increase, based on the modification of the heat increment and metabolizable energy values as determined for maintenance, to conform to the ratios of the corresponding values (heat increment and metabolizable energy) of the mixed ration, for maintenance, to the same for body increase; (6) the use of a new procedure in determining the excess of energy expenditure of standing as compared with lying, in the computation of the heat production to conform to a standard day as to standing and lying, this factor being based on a recent and much improved consideration of this matter (*12*); and (7) to meet a special situation the average of the directly observed and the computed heat production (balance method) was used, in all instances in which both were available, in studying the energy metabolism as related to the plane of nutrition.

These several procedures are discussed in detail later in this paper, each in connection with the computations in which it is involved.

EXPERIMENTAL SUBJECTS

The steers used, which are designated Nos. 36 and 47, were unusually good subjects. They were of the same breed—Aberdeen-Angus—and had been thoroughly accustomed to experiments in the calorimeter through previous experience.

Steer No. 36 was calved on October 10, and steer No. 47 on August 29, both in the year 1924. During these experiments steer No. 36 was between 25 and 31 months, and steer No. 47 between 27 and 33 months, of age.

The steers were in good flesh, somewhat more than half fat, and came into the experimental program from a summer at pasture without grain feed.

They were of almost identical live weight. The average daily weight of each varied appreciably, from period to period, as the different planes of nutrition affected the "fill" and the true body weight, but the two steers agreed with each other in average daily weights in the several periods much more closely than ordinarily do consecutive daily weights of an individual steer.

EXPERIMENTAL DATA

The main foundation data of the experiments, as well as the corrections and computations necessary for their comparison and interpretation, are to be found in Tables 2 to 19, inclusive, while the derived final results are to be found in Tables 20 to 23. These tables will be discussed in numerical order.

TABLE 2.—*Digestibility of rations*

Period No.	Animal No.	Item	Dry matter	Organic matter	Crude protein	Crude fiber	Ether extract	N-free extract	Carbon	Energy	Nitrogen
			Gms.	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Gms.*	*Cals.*	*Gms.*
1	47	Salt	30								
		Alfalfa hay	3,721	3,372.1	587.5	1,252.0	56.1	1,476.5	1,698.4	16,665.2	94.0
		Corn meal	3,663	3,605.7	411.2	80.4	148.1	2,966.0	1,689.0	16,701.9	65.8
		Total fed	7,414	6,977.8	998.7	1,332.4	204.2	4,442.5	3,387.4	33,367.1	159.8
		Feces	2,016	1,842.7	363.1	743.5	79.8	656.3	1,001.3	10,142.1	58.1
		Digestibility (per cent)	72.8	73.6	63.6	44.2	60.9	85.2	70.4	69.6	63.6
2	36	Salt	30								
		Alfalfa hay	3,545	3,212.6	559.7	1,192.8	53.4	1,406.7	1,618.1	15,877.0	89.5
		Corn meal	3,492	3,437.4	392.0	76.6	141.2	2,827.6	1,610.2	15,922.2	62.7
		Total fed	7,067	6,650.0	951.7	1,269.4	194.6	4,234.3	3,228.3	31,799.2	152.2
		Feces	1,973	1,797.1	360.2	720.3	76.8	639.8	965.0	9,794.3	57.6
		Digestibility (per cent)	72.1	73.0	62.2	43.3	60.5	84.9	70.1	69.2	62.2
3	47	Salt	30								
		Alfalfa hay	2,832	2,566.4	447.1	952.9	42.7	1,123.7	1,292.6	12,683.7	71.5
		Corn meal	2,785	2,741.4	312.0	61.1	112.6	2,255.1	1,284.2	12,698.5	50.0
		Total fed	5,647	5,307.8	759.7	1,014.0	155.3	3,378.8	2,576.8	25,382.2	121.5
		Feces	1,419	1,292.8	254.0	549.4	54.6	434.8	688.5	6,940.3	40.6
		Digestibility (per cent)	74.9	75.6	66.6	45.8	64.8	87.1	73.3	72.7	66.6
4	36	Salt	30								
		Alfalfa hay	2,698	2,445.0	428.0	907.8	40.7	1,070.5	1,231.5	12,083.5	68.2
		Corn meal	2,655	2,613.5	296.0	58.3	107.4	2,149.8	1,224.2	12,105.8	47.7
		Total fed	5,383	5,058.5	724.0	966.1	148.1	3,220.3	2,455.7	24,189.3	115.9
		Feces	1,371	1,232.6	247.0	531.9	58.0	395.7	659.9	6,668.8	39.5
		Digestibility (per cent)	74.5	75.6	65.9	44.9	60.8	87.7	73.1	72.4	65.9
5	47	Salt	30								
		Alfalfa hay	943	854.6	148.9	317.3	14.2	374.2	430.4	4,223.4	23.8
		Corn meal	920	905.7	95.3	20.5	35.8	754.1	426.1	4,193.8	15.2
		Total fed	1,893	1,760.3	244.2	337.8	50.0	1,128.3	856.5	8,417.2	39.0
		Feces	456	410.8	73.0	200.7	18.6	118.5	222.2	2,262.3	11.7
		Digestibility (per cent)	75.9	76.7	70.1	40.6	62.8	89.5	74.1	73.1	70.1
6	36	Salt	30								
		Alfalfa hay	949	860.0	149.8	319.3	14.3	376.6	433.2	4,250.3	24.0
		Corn meal	936	921.4	96.9	20.8	36.4	767.3	433.5	4,266.7	15.5
		Total fed	1,915	1,781.4	246.7	340.1	50.7	1,143.9	866.7	8,517.0	39.5
		Feces	467	424.3	71.1	220.3	17.9	115.0	229.1	2,326.5	11.4
		Digestibility (per cent)	75.6	76.2	71.2	35.2	64.7	89.9	73.6	72.7	71.2

TABLE 2.—*Digestibility of rations*—Continued

Period No.	Animal No.	Item	Dry matter	Organic matter	Crude protein	Crude fiber	Ether extract	N-free extract	Carbon	Energy	Nitrogen
7	47	Salt	30								
		Alfalfa hay	1,911	1,731.8	301.7	643.0	28.8	758.3	872.3	8,558.8	48.3
		Corn meal	1,879	1,849.7	194.6	41.8	73.1	1,540.2	870.3	8,565.3	31.1
		Total fed	3,820	3,581.5	496.3	684.8	101.9	2,298.5	1,742.6	17,124.1	79.4
		Feces	897	812.0	148.2	378.3	38.1	247.4	438.7	4,399.0	23.7
		Digestibility (per cent)	76.5	77.3	70.1	44.8	62.6	89.2	74.8	74.3	70.1
6	36	Salt	30								
		Alfalfa hay	1,902	1,723.6	300.3	640.0	28.7	754.6	868.1	8,518.5	48.0
		Corn meal	1,860	1,831.0	102.6	41.3	72.4	1,524.7	861.5	8,478.7	30.8
		Total fed	3,792	3,554.6	402.9	681.3	101.1	2,279.3	1,729.6	16,997.2	78.8
		Feces	900	806.5	144.1	380.8	36.1	245.5	441.5	4,466.0	23.0
		Digestibility (per cent)	76.3	77.3	70.8	44.1	64.3	89.2	74.5	73.7	70.8
9	47	Salt	30								
		Alfalfa hay	5,771	5,229.9	911.1	1,941.8	87.0	2,290.0	2,634.1	25,846.6	145.8
		Total fed	5,801	5,229.9	911.1	1,941.8	87.0	2,290.0	2,634.1	25,846.6	145.8
		Feces	2,339	2,117.5	297.5	1,111.2	84.6	624.2	1,130.2	11,244.8	47.6
		Digestibility (per cent)	59.7	59.5	67.3	42.8	2.8	72.7	57.1	56.5	67.3
10	36	Salt	30								
		Alfalfa hay	5,763	5,222.6	909.9	1,939.1	86.8	2,286.8	2,630.5	25,810.7	145.6
		Total fed	5,793	5,222.6	909.9	1,939.1	86.8	2,286.8	2,630.5	25,810.7	145.6
		Feces	2,334	2,109.1	30.1	1,091.6	84.7	631.7	1,130.1	11,280.9	48.2
		Digestibility (per cent)	59.7	59.6	66.9	43.7	2.4	72.4	57.0	56.3	66.9

The digestibility of the rations, as set forth in Table 2, is the usual apparent digestibility, representing the difference between the amounts of constituents in feed and feces.

The considerable length of the digestion periods and the high degree of regularity of the treatment given the animals were reflected in an unusually satisfactory regularity in the elimination of excreta; and the fact that the composition of the ration was the same in all but two periods, Nos. 9 and 10, constituted a favorable basis for criticism of results obtained. The digestion coefficients, therefore, are good figures of their sort.

The more noteworthy coefficients are those representing the rations of alfalfa hay alone, fed in periods 9 and 10, the digestibility of the nitrogen-free extract being decidedly low (72.4 to 72.7 per cent for the alfalfa hay, as compared with 84.7 to 89.9 per cent for the mixed ration) and that of the ether extract being almost negligible (2.4 to 2.8 per cent for the alfalfa hay, as compared with 60.5 to 64.8 per cent for the mixed ration).

These data call attention to the facts that ether extract includes a very great diversity of chemical compounds and that the apparent digestibility of ether extract may be a highly deceptive observation—especially under conditions such that the ether extract of the feces is largely of metabolic origin—thus not a feed residue.

The digestibility of the rations in relation to the plane of nutrition will be discussed in connection with the partition of the gross energy.

The quantities of carbon dioxide, water vapor, and methane given off by the experimental subjects during each calorimeter day are recorded in Table 3 as an exhibit of the degree of regularity, and therefore of reliability, characterizing these data which enter in important ways into the computation of the heat production and the metabolizable energy.

TABLE 3.—*Carbon dioxide, water vapor, and methane eliminated per day*

Animal No.	Period No.	Calorimeter day	Elimination of—				
			CO_2	C as CO_2	H_2O	CH_4	C as CH_4
			Grams	*Grams*	*Grams*	*Grams*	*Grams*
Steer 47	1	First	5,843.55	1,593.54	5,496.94	177.47	132.81
		Second	5,976.87	1,629.89	6,057.24	186.26	139.39
		Third	6,029.48	1,644.24	7,159.39	185.88	139.10
		Average	5,949.97	1,622.56	6,237.85	183.20	137.10
Steer 36	2	First	5,812.48	1,585.06	5,864.28	160.89	120.41
		Second	6,023.82	1,642.42	7,313.00	164.60	123.16
		Third	6,033.10	1,645.23	6,796.25	162.14	121.34
		Average	5,956.13	1,624.24	6,657.84	162.54	121.64
Steer 47	3	First	5,053.36	1,378.05	6,329.57	140.08	104.83
		Second	5,075.37	1,384.05	6,566.50	142.17	106.39
		Third	5,122.79	1,396.98	6,643.97	140.42	105.08
		Average	5,083.84	1,386.36	6,513.35	140.89	105.43
Steer 36	4	First	5,094.79	1,389.35	6,468.77	141.30	105.74
		Second	4,975.13	1,356.72	6,508.00	134.73	100.83
		Third	5,044.07	1,375.52	6,678.48	137.97	103.25
		Average	5,038.00	1,373.86	6,551.75	138.00	103.27
Steer 47	5	First	2,901.87	791.34	3,478.84	65.20	48.79
		Second	2,852.54	777.89	3,356.94	61.21	45.81
		Third	2,919.17	796.06	3,490.29	59.48	44.51
		Average	2,891.19	788.43	3,442.02	61.96	46.37
Steer 36	6	First	3,034.36	827.47	3,542.92	66.91	50.08
		Second	3,027.13	825.50	3,231.74	62.58	46.83
		Third	3,015.94	822.45	3,230.14	57.43	42.98
		Average	3,025.81	825.14	3,334.93	62.31	46.63
Steer 47	7	First	3,932.39	1,072.36	4,389.37	111.85	83.70
		Second	3,859.75	1,052.55	4,273.07	107.77	80.65
		Third	3,922.04	1,069.54	4,415.26	106.12	79.42
		Average	3,904.73	1,064.82	4,359.23	108.58	81.26
Steer 36	8	First	4,050.14	1,104.47	4,750.43	110.98	83.05
		Second	4,040.16	1,101.75	4,434.02	111.23	83.24
		Third	4,010.92	1,093.78	4,280.65	110.90	82.99
		Average	4,033.74	1,100.00	4,488.37	111.04	83.09
Steer 47	9	First	4,498.91	1,226.85	6,568.68	127.35	95.31
		Second	4,480.55	1,221.85	6,145.39	124.12	92.88
		Average	4,489.73	1,224.35	6,357.04	125.74	94.10
Steer 36	10	First	4,762.04	1,298.61	5,860.43	122.63	91.77
		Second	4,695.51	1,280.47	5,800.42	124.34	93.05
		Third	4,762.95	1,298.86	5,875.55	125.40	93.85
		Average	4,740.17	1,292.65	5,845.47	124.12	92.89

Calorimeter period No. 9 was terminated during the third day on account of refusal of feed; therefore the data are complete for only two days during this period. In all other cases the data cover three full days.

The agreement between the data for the individual days of each period was remarkably good, and in no complete day were the data considered unfit for inclusion in the computation of averages.

The water vapor was not measured in periods 1 and 2 on account of the fact that the heat production was not determined by the direct method during these periods; the data for water vapor, therefore,

not being needed, as usual, for the determination of the latent heat of water vapor, and for the water balance.

Preliminary to the computation of the balance of matter an important correction, based on the nitrogen balance, was applied to the potential energy of the urine and of the body balance of protein.

In order to obtain a correct value of the metabolizable energy of the ration it is necessary to consider the gross energy of the body gain as metabolizable, except for that portion of the energy of the protein gained which would appear unoxidized in the urine if the protein gained had all been katabolized. From the point of view of energy metabolism, therefore, a part of the energy of protein stored is not metabolizable.

Likewise, in case of a loss of protein from the body, the metabolizable energy of this protein is less than its gross energy by that quantity appearing unoxidized in the urine.

The metabolizable energy of protein either gained or lost, therefore, is less than its gross energy by the amount of the energy of the urinary constituents which would result from the katabolism of this protein.

The amount of such minus corrections of the metabolizable energy, on account of the nonmetabolizable fraction of the protein gained or lost, is used as a plus correction of the energy of the urine in case of body gain of protein, and as a minus correction of the same in case of body loss of protein.

The gross or potential energy of body protein is obtained by multiplying its amount in grams by the factor 5.7; and the correction for the nonmetabolizable fraction of such protein is computed by multiplying the body gain or loss of nitrogen, in grams, by the factor 7.45.

TABLE 4.—*Energy of the urine and of the protein corrected for the incomplete oxidation of protein gained or lost*

Period No.	Balance of N	Correction (N×7.45)	Energy of urine		Energy of protein	
			Uncorrected for N equilibrium	Corrected	Uncorrected (grams protein×5.7)	Corrected
	Grams	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
1	+14.6	108.8	1,146.4	1,255.2	499.3	390.5
2	+11.2	83.4	1,133.6	1,217.0	383.0	299.6
3	+6.7	49.9	996.8	1,046.7	229.1	179.2
4	+5.5	41.0	1,001.7	1,042.7	188.1	147.1
5	−14.0	104.3	536.7	432.4	478.8	374.5
6	−13.8	102.8	566.0	403.2	472.0	369.2
7	+7.8	58.1	710.2	768.3	266.8	208.7
8	+8.7	64.8	737.2	802.0	297.5	232.7
9	+8.7	64.8	1,243.3	1,308.1	297.5	232.7
10	+8.4	62.6	1,260.9	1,323.5	287.3	224.7

The corrected values as given in Table 4 were employed in the computation of the balance of energy, as in Table 5.

TABLE 5.—*Balance of matter and energy per day*

Item	Dry matter	Water	Nitrogen	Carbon	Energy
PERIOD 1, STEER NO. 47					
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*
Alfalfa hay	3,721.0		94.0	1,698.4	16,665.2
Corn meal	3,663.0		65.8	1,689.0	16,701.9
Total	7,384.0		159.8	3,387.4	33,367.1
Outgo:					
Feces	2,016.0		58.1	1,001.3	10,142.1
Urine			87.1	129.6	1,255.2
Methane	183.2			137.1	2,444.6
Carbon dioxide	5,950.0			1,622.5	
Metabolizable:					
Income minus urine, feces, and methane					19,525.2
Body balances:					
Fat	+589.3			+450.9	+5,598.4
Protein	+87.6		+14.6	+46.0	+390.5
Computed heat production					13,536.3
PERIOD 2, STEER NO. 36					
Income:					
Alfalfa hay	3,545.0		89.5	1,618.1	15,877.0
Corn meal	3,492.0		62.7	1,610.2	15,922.2
Total	7,037.0		152.2	3,228.3	31,799.2
Outgo:					
Feces	1,973.0		57.6	965.0	9,794.3
Urine			83.4	125.3	1,217.0
Methane	162.5			121.6	2,168.4
Carbon dioxide	5,956.1			1,624.2	
Metabolizable:					
Income minus urine, feces, and methane					18,619.5
Body balances:					
Fat	+466.5			+356.9	+4,431.8
Protein	+67.2		+11.2	+35.3	+299.6
Computed heat production					13,888.1
PERIOD 3, STEER No. 47					
Income:					
Alfalfa hay	2,832.0	410	71.5	1,292.6	12,683.7
Corn meal	2,785.0	472	50.0	1,284.2	12,698.5
Water		15,713			
Total	5,617.0	16,595	121.5	2,576.8	25,382.2
Outgo:					
Feces	1,419.0	4,389	40.6	688.5	6,940.3
Urine		7,935	74.2	110.6	1,046.7
Methane	140.9			105.4	1,880.2
Carbon dioxide	5,083.9			1,386.4	
Water vapor		6,514			
Metabolizable:					
Income minus urine, feces, and methane					16,515.0
Body balances:					
Fat	+346.1			+264.8	+3,288.0
Protein	+40.2		+6.7	+21.1	+179.2
Water		−2,243			
Computed heat production					12,047.8
Observed heat production					11,692.9

TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 4, STEER No. 36

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Cals*
Income:					
Alfalfa hay	2,698.0	385	68.2	1,231.5	12,083.5
Corn meal	2,655.0	438	47.7	1,224.2	12,105.8
Water		12,723			
Total	5,353.0	13,546	115.9	2,455.7	24,189.3
Outgo:					
Feces	1,371.0	5,689	39.5	659.9	6,668.8
Urine		5,487	70.9	109.8	1,042.7
Methane	138.0			103.3	1,841.5
Carbon dioxide	5,038.0			1,373.9	
Water vapor		6,552			
Metabolizable:					
Income minus urine, feces, and methane					14,636.3
Body balances:					
Fat	+250.3			+191.5	+2,377.9
Protein	+33.0		+5.5	+17.3	+147.1
Water		−4,182			
Computed heat production					12,111.3
Observed heat production					11,854.1

PERIOD 5, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:					
Alfalfa hay	943.0	121	23.8	430.4	4,223.4
Corn meal	920.0	173	15.2	426.1	4,193.8
Water		6,237			
Total	1,863.0	6,531	39.0	856.5	8,417.2
Outgo:					
Feces	456.0	853	11.7	222.2	2,262.3
Urine		4,351	41.3	55.1	432.4
Methane	62.0			46.4	827.3
Carbon dioxide	2,891.2			788.4	
Water vapor		3,442			
Metabolizable:					
Income minus urine, feces, and methane					4,895.2
Body balances:					
Fat	−276.4			−211.5	−2,625.8
Protein	−84.0		−14.0	−44.1	−374.5
Water		−2,115			
Computed heat production					7,895.5
Observed heat production					7,754.5

PERIOD 6, STEER NO. 36

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:					
Alfalfa hay	949.0	134	24.0	433.2	4,250.3
Corn meal	936.0	157	15.5	433.5	4,266.7
Water		9,073			
Total	1,885.0	9,364	39.5	866.7	8,517.0
Outgo:					
Feces	467.0	1,044	11.4	229.1	2,326.5
Urine		4,926	41.9	57.6	463.2
Methane	62.3			46.6	831.3
Carbon dioxide	3,025.8			825.2	
Water vapor		3,335			
Metabolizable:					
Income minus urine, feces, and methane					4,896.0
Body balances:					
Fat	−324.5			−248.3	−3,082.8
Protein	−82.8		−13.8	−43.5	−369.2
Water		+59			
Computed heat production					8,348.0
Observed heat production					8,155.8

TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 7, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Cals.*
Income:					
Alfalfa hay	1,911.0	225	48.3	872.3	8,558.8
Corn meal	1,879.0	313	31.1	870.3	8,565.3
Water		10,693			
Total	3,790.0	11,231	79.4	1,742.6	17,124.1
Outgo:					
Feces	807.0	2,795	23.7	438.7	4,399.0
Urine		5,650	47.9	77.7	768.3
Methane	108.6			81.3	1,449.2
Carbon dioxide	3,904.7			1,064.8	
Water vapor		4,359			
Metabolizable:					
Income minus urine, feces, and methane					10,507.6
Body balances:					
Fat	+72.5			+55.5	+688.8
Protein	+46.8		+7.8	+24.6	+208.7
Water		−1,573			
Computed heat production					9,610.1
Observed heat production					9,982.8

PERIOD 8, STEER NO. 36

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:					
Alfalfa hay	1,902.0	257	48.0	868.1	8,518.5
Corn meal	1,860.0	332	30.8	861.5	8,478.7
Water		9,119			
Total	3,762.0	9,708	78.8	1,729.6	16,997.2
Outgo:					
Feces	900.0	3,629	23.0	441.5	4,466.0
Urine		5,035	47.1	78.8	802.0
Methane	111.0			83.1	1,481.2
Carbon dioxide	4,033.7			1,100.0	
Water vapor		4,488			
Metabolizable:					
Income minus urine, feces, and methane					10,248.0
Body balances:					
Fat	−1.6			−1.2	−15.2
Protein	+52.2		+8.7	+27.4	+232.7
Water		−3,444			
Computed heat production					10,030.5
Observed heat production					9,839.7

PERIOD 9, STEER NO. 47

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:					
Alfalfa hay	5,771.0	677	145.8	2,634.1	25,846.6
Water		12,445			
Total	5,771.0	13,122	145.8	2,634.1	25,846.6
Outgo:					
Feces	2,339.0	6,777	47.6	1,130.2	11,244.8
Urine		8,464	89.5	141.0	1,308.1
Methane	125.8			94.1	1,678.7
Carbon dioxide	4,489.6			1,224.4	
Water vapor		6,357			
Metabolizable:					
Income minus urine, feces, and methane					11,615.0
Body balances:					
Fat	+22.2			+17.0	+210.9
Protein	+52.2		+8.7	+27.4	+232.7
Water		−8,496			
Computed heat production					11,171.4
Observed heat production					11,254.6

TABLE 5.—*Balance of matter and energy per day*—Continued

PERIOD 10, STEER NO. 36

Item	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*
Alfalfa hay	5,763.0	613	145.6	2,630.5	25,810.7
Water		17,410			
Total	5,763.0	18,023	145.6	2,630.5	25,810.7
Outgo:					
Feces	2,334.0	7,491	48.2	1,130.1	11,280.9
Urine		7,689	89.0	143.5	1,323.5
Methane	124.1			92.0	1,656.0
Carbon dioxide	4,740.2			1,292.7	
Water vapor		5,846			
Metabolizable:					
Income minus urine, feces, and methane					11,550.3
Body balances:					
Fat	−72.1			−55.2	−685.0
Protein	+50.4		+8.4	+26.5	+224.7
Water		−3,003			
Computed heat production					12,010.6
Observed heat production					11,635.0

It is helpful in grasping the significance of this correction to realize that it distinguishes, in any case, between the gross energy of protein and that portion of its energy which is available to the animal.

In Table 5 are given the average daily income and outgo of water, nitrogen, carbon, and energy; the metabolizable energy of the ration; and the body balances of fat, protein, energy, and water; also, for convenience in the use of these data, the amounts of both the computed and the observed heat production, in all but two periods, are given.

It will be noted that in all cases in which there are values for both the observed and the computed heat production, except in period 9, the computed value is slightly higher than the observed, as measured by direct calorimetry. The cause of this difference was not determined, and no satisfactory basis for choice between the two values was established. After carefully weighing the various possibilities of error in both methods of determination of the heat production, it was concluded that, pending the accomplishment of certain improvements in the mechanical accessories of the calorimetric equipment, the values of the computed and the directly observed heat production should be averaged.

Three important corrections of the heat production were made preparatory to its final use.

The first, shown in Table 6, is a correction of the observed heat production, based on the body gain or loss of protein, fat, and water, for the purpose of correcting for the gain or loss of sensible heat by the body of the animal while in the calorimeter. This correction is necessitated by the fact that in case of gain of these components heat is stored which has not been measured, and, in case of loss, heat is liberated as these components cool to the chamber temperature, the measured heat being in excess by the quantity so lost.

In Table 6 is also given the heat emission, by radiation and conduction, as observed, for subperiods of 12 hours each, there being six such intervals in each experimental period. A comparison of these

TABLE 6.—*Heat emission and heat production per day*

Animal No.	Period No.	Calorimeter days	Sub-period No.	Heat emission: By radiation and conduction	Heat emission: As latent heat of water vapor	Heat emission: Total	Correction for body gain	Heat production
				Calories	*Calories*	*Calories*	*Calories*	*Calories*
			1	3,840.89				
			2	4,324.13				
		First		8,165.02	3,505.53	11,670.55		
			3	3,770.85				
			4	4,283.24				
47	3	Second		8,060.09	3,645.32	11,705.41		
			5	4,002.05				
			6	4,154.35				
		Third		8,157.30	3,691.02	11,848.32		
		Daily average		8,127.47	3,613.96	11,741.43	−48.6	11,692.9
			1	4,181.07				
			2	4,204.62				
		First		8,385.69	3,763.47	12,149.16		
			3	3,967.49				
			4	4,161.41				
36	4	Second		8,128.90	3,719.36	11,848.26		
			5	3,963.57				
			6	4,186.72				
		Third		8,150.29	3,707.84	11,858.13		
		Daily average		8,221.63	3,730.22	11,951.85	−97.7	11,854.1
			1	2,918.25				
			2	2,826.33				
		First		5,744.58	2,045.56	7,790.14		
			3	2,924.36				
			4	2,827.10				
47	5	Second		5,751.46	1,973.88	7,725.34		
			5	2,851.06				
			6	2,997.63				
		Third		5,848.69	2,052.29	7,900.98		
		Daily average		5,781.58	2,023.91	7,805.49	−50.9	7,754.5
			1	3,009.35				
			2	3,090.69				
		First		6,109.04	2,083.24	8,192.28		
			3	2,926.27				
			4	3,214.15				
31	6	Second		6,140.42	1,900.26	8,040.68		
			5	3,070.11				
			6	3,276.77				
		Third		6,346.88	1,899.32	8,246.20		
		Daily average		6,198.78	1,960.94	8,159.72	−3.9	8,155.8
			1	3,407.64				
			2	3,443.52				
		First		6,851.16	2,580.95	9,432.11		
			3	3,429.89				
			4	3,370.37				
47	7	Second		6,800.26	2,512.57	9,312.83		
			5	3,437.63				
			6	3,468.72				
		Third		6,906.35	2,596.18	9,502.53		
		Daily average		6,852.59	2,563.23	9,415.82	−33.0	9,382.8
			1	3,654.87				
			2	3,684.66				
		First		7,339.53	2,793.25	10,132.78		
			3	3,592.45				
			4	3,684.71				
36	8	Second		7,277.16	2,607.20	9,884.36		
			5	3,618.55				
			6	3,590.41				
		Third		7,208.96	2,517.02	9,725.98		
		Daily average		7,275.22	2,639.15	9,914.37	−74.7	9,839.7

TABLE 6.—*Heat emission and heat production per day*—Continued

Animal No.	Period No.	Calorimeter days	Subperiod No.	Heat emission: By radiation and conduction	Heat emission: As latent heat of water vapor	Heat emission: Total	Correction for body gain	Heat production
				Calories	*Calories*	*Calories*	*Calories*	*Calories*
47	9		1	4,386.29				
			2	4,161.40				
		First		8,547.69	3,088.58	11,636.27		
			3	4,134.76				
			4	4,082.61				
		Second		8,217.37	3,026.96	11,244.33		
		Daily average		8,382.53	3,057.77	11,440.30	−185.7	11,254.6
36	10		1	4,086.53				
			2	4,365.98				
		First		8,452.51	3,445.99	11,898.50		
			3	3,907.65				
			4	4,149.88				
		Second		8,057.53	3,410.64	11,468.17		
			5	3,921.35				
			6	4,360.02				
		Third		8,281.37	3,454.82	11,736.19		
		Daily average		8,263.80	3,437.15	11,700.95	−66.0	11,635.0

subperiod data is not without interest, as revealing the character, as to uniformity, of the data entering into the determination of the average daily heat production. No close comparison of these subperiod values can be made, however, especially because they represent different proportionate intervals of time spent in the standing and the lying positions. The latent heat of water vapor is given, not for subperiods, but for experimental days.

The second and third of the above-mentioned corrections were applied to the average of the final observed and computed values for the heat production. These were a correction (Table 7) based on the duration of time spent by the animal in the standing and the lying positions, and the difference in energy expenditure of standing and lying—for use in the computation of the heat production of the standard day, in which the animal stood and lay down 12 hours each; and a correction (Table 18) for difference in the maintenance requirement of net energy, in the several experimental periods, due to difference in live weight, this correction being devised to permit the comparison of the heat production of the animal on different planes of nutrition.

In computing the standing and lying correction the difference between the aggregate of intervals of time spent by the animal in the standing position, as compared with the 12-hour standard, is multiplied by a factor representing the amount by which the energy cost of standing, per unit of time, is greater than that of lying—this factor being adjusted to the live weight of the animal. The derivation of this factor is discussed in detail in a recent paper in this Journal (*12*) by Forbes, Kriss, Braman, and associates.

It should be noted that this correction, as determined for the steers used in this series of experiments, is materially greater than that used (*13*) for the same purpose in other recent papers from this

institute. It seems that, for the present at least, determinations of this correction will have to be made in connection with each exper*imental program.*

TABLE 7.—*Correction of heat production to a standard day of 12 hours standin and 12 hours lying*

Period No.	Steer No.	Time spent standing	Difference from 12 hours	Factor	Correction	Heat production: Uncorrected	Heat production: Corrected	Heat observed or computed
		Hours	*Hours*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	
1	47	8.5	3.5	73.4	256.9	13,536.3	13,793.2	Computed.
2	36	13.4	1.4	72.9	−102.1	13,888.1	13,786.0	Do.
3	47	7.9	4.1	74.7	306.3	11,692.9	11,999.2	Observed.
						12,047.8	12,354.1	Computed.
4	36	10.4	1.6	74.0	118.4	11,851.1	11,972.5	Observed.
						12,111.3	12,229.7	Computed.
5	47	7.5	4.5	71.7	322.7	7,754.5	8,077.2	Observed.
						7,895.5	8,218.2	Computed.
6	36	8.9	3.1	71.2	220.7	8,155.8	8,376.5	Observed.
						8,348.0	8,568.7	Computed.
7	47	6.9	5.1	73.2	373.3	9,382.8	9,756.1	Observed.
						9,610.1	9,983.4	Computed.
8	36	9.5	2.5	72.7	181.8	9,839.7	10,021.5	Observed.
						10,030.5	10,212.3	Computed.
9	47	7.3	4.7	75.3	353.9	11,254.6	11,608.5	Observed.
						11,171.4	11,525.3	Computed.
10	36	12.8	.8	75.5	−60.4	11,635.0	11,574.6	Observed.
						12,010.6	11,950.2	Computed.

DETERMINATION OF THE FASTING KATABOLISM AS A MEASURE OF THE MAINTENANCE REQUIREMENT OF NET ENERGY

The directly determined fasting katabolism was adopted by thi institute as the measure of the maintenance requirement of net energ of cattle in 1925, this departure from the earlier procedure (*1*) and th data upon which it rested being published by Forbes in a paper rea before the American Society of Animal Production (*6*).

Further notice of the adoption of this measure—new as applied t cattle—was given by Forbes in Science (*5*); and a more extende study of the same matter, with additional experimental data, wa published by Forbes, Kriss, and Braman in 1927 (*11*).

In the nature of the case, however, this measure can not be regarde as an absolute value; and its use in studies of the nutrition of cattl depends on the exact method by which it (the directly determined fasting katabolism) is determined.

Since the date of adoption of this measure, therefore, the conventional standardization of procedure in its determination has bee a continuing problem at this institute, and the present discussio may be considered—apart from its relation to the main study i hand—as a further contribution to the subject of standardizatio of this determination.

The principal problem in regard to this measurement is as to th exact conditions under which the heat production of the animal is t be considered representative of true fast—a problem resulting, in th main, from the facts as to the complicated anatomy and physiolog of the ruminant alimentary tract—a situation which was discussed a some length in the last paper (*11*) above referred to.

As a background condition it is the practice at this institute t determine the fasting katabolism only after a period of feeding on

plane of energy equilibrium, of sufficient length presumably to have accomplished the adjustment of the animal to this regimen. Under these conditions, if there is a carrying over of any habit or influence as to energy metabolism from the previous state of nutrition into the days of fast during which the fasting katabolism is measured, then this carrying over will be, in all cases, at least of similar origin and degree.

In the present experiments the sequence of treatments was such that both steers were on a plane of approximate energy equilibrium for 40 days immediately before the fasting periods; and during the last day of feeding only grain was given, roughage being withheld, with the idea that this procedure might assist in freeing the paunch from feed.

The steers were then further prepared for the measurement of the fasting katabolism by the administration of a physic. The preparation used was composed of sodium sulphate 12 parts, capsicum 2 parts, ginger 2 parts, and gamboge 1 part; and the treatment was two ½-pound doses, separated by a two-hour interval, on two successive mornings.

TABLE 8.—*Carbon dioxide, oxygen, and nitrogen in ingoing air during fast*

Animal, period, and subperiod Nos.	Aliquot, ingoing air	Carbon dioxide in aliquot	Carbon dioxide in aliquot	Carbon dioxide	Oxygen	Nitrogen
	Liters	*Grams*	*Liters*	*Per cent*	*Per cent*	*Per cent*
Steer No. 47, period 11:						
Subperiod 1	446.75	0.2899	0.1467	0.033	[a] 20.935	79.032
Subperiod 2						
Subperiod 3	449.44	.2704	.1368	.030	20.935	79.035
Subperiod 4	447.36	.2800	.1417	.032	20.935	79.033
Subperiod 5				.031	20.935	79.034
Subperiod 6	444.66	.2648	.1340	.030	20.935	79.035
Subperiod 7	449.15	.2621	.1326	.030	20.935	79.035
Subperiod 8	447.44	.2629	.1330	.030	20.935	79.035
Steer No. 36, period 12:						
Subperiod 1	435.22	.2750	.1391	.032	20.929	79.039
Subperiod 2	431.73	.2786	.1409	.033	20.937	79.030
Subperiod 3	433.69	.2837	.1435	.033	20.932	79.035
Subperiod 4	433.33	.2782	.1407	.032	20.936	79.032
Subperiod 5	425.82	.2752	.1392	.033	20.940	79.027
Subperiod 6	437.77	.2847	.1440	.033	20.935	79.032
Subperiod 7	438.30	.2709	.1370	.031	20.936	79.033
Subperiod 8	435.42	.2748	.1390	.032	20.934	79.034

[a] Average of determinations for period 12.

TABLE 9.—*Carbon dioxide and oxygen in outcoming air during fast*

Animal, period, and subperiod Nos.	Outcoming air	Carbon dioxide	Carbon dioxide	Carbon dioxide	Oxygen
	Liters	*Grams*	*Liters*	*Per cent*	*Per cent*
Steer No. 47, period 11:					
Subperiod 1	323,282.8	1,463.8	740.5	0.229	20.678
Subperiod 2					
Subperiod 3	324,112.5	1,316.4	666.0	.205	20.698
Subperiod 4	324,399.2	1,380.0	698.1	.215	20.696
Subperiod 5	322,125.9	1,311.6	663.5	.206	20.719
Subperiod 6	322,342.1	1,332.1	673.9	.209	20.704
Subperiod 7	326,186.6	1,319.9	667.7	.205	20.706
Subperiod 8	326,640.2	1,309.6	662.5	.203	20.709
Steer No. 36, period 12:					
Subperiod 1	321,541.7	1,503.6	760.7	.237	20.685
Subperiod 2	321,235.3	1,479.1	748.3	.233	20.690
Subperiod 3	321,221.6	1,415.0	715.8	.223	20.692
Subperiod 4	322,791.1	1,392.6	704.5	.218	20.693
Subperiod 5	324,127.9	1,347.6	681.8	.210	20.700
Subperiod 6	324,391.1	1,370.1	693.1	.214	20.702
Subperiod 7	323,775.2	1,419.6	718.2	.222	20.692
Subperiod 8	324,485.1	1,341.4	678.6	.209	20.700

16107—28——2

TABLE 10.—*Urinary nitrogen per 12-hour subperiod, and carbon dioxide, oxygen, and energy equivalents, during fast*

Animal and period Nos.	Average urinary nitrogen per 12-hour subperiod	Carbon dioxide equivalent (N×4.75)	Oxygen equivalent (N×5.94)	Energy equivalent (N×26.51)
	Grams	*Liters*	*Liters*	*Cals.*
Steer 47, period 11	18.7	88.8	111.1	495.7
Steer 36, period 12	21.1	100.2	125.3	559.4

TABLE 11.—*Heat production computed from the respiratory quotient, compared with the observed heat production (not corrected to the standard day), during fast*

Animal, period, and subperiod Nos.	Non-protein R. Q.	Corrected value per liter O_2	Total non-protein oxygen	Computed heat production: Non-protein	Computed heat production: Protein	Computed heat production: Total	Observed heat production	Computed observed heat production
Steer No. 47, period 11.		*Cals.*	*Liters*	*Cals.*	*Cals.*	*Cals.*	*Cals*	*Per cent*
Subperiod 1	0.706	4.686	772.0	3,617.6	495.7	4,113.3	4,533.0	90.7
Subperiod 2								
Subperiod 3	.673	4.686	710.3	3,328.5	495.7	3,824.2	3,703.5	103.3
Subperiod 4	.709	4.688	712.3	3,339.3	495.7	3,835.0	3,817.4	100.5
Subperiod 5	.760	4.759	619.6	2,948.7	495.7	3,444.4	3,630.2	94.9
Subperiod 6	.720	4.702	677.9	3,187.5	495.7	3,683.2	3,734.6	98.6
Subperiod 7	.706	4.686	682.5	3,198.2	495.7	3,693.9	3,616.7	102.1
Subperiod 8	.708	4.687	673.0	3,154.4	495.7	3,650.1	3,684.9	99.1
Steer No. 36, period 12:								
Subperiod 1	.807	4.809	692.5	3,330.2	559.4	3,889.6	4,315.6	90.1
Subperiod 2	.780	4.776	695.1	3,319.8	559.4	3,879.2	4,076.6	95.2
Subperiod 3	.741	4.728	688.1	3,253.3	559.4	3,812.7	3,993.1	95.5
Subperiod 4	.707	4.686	707.8	3,316.8	559.4	3,876.2	3,881.4	99.9
Subperiod 5	.670	4.685	706.7	3,311.6	559.4	3,871.0	3,731.7	103.7
Subperiod 6	.721	4.703	675.3	3,175.9	559.4	3,735.3	3,781.7	98.8
Subperiod 7	.732	4.717	707.9	3,339.2	559.4	3,898.6	4,071.3	95.8
Subperiod 8	.694	4.686	683.0	3,200.5	559.4	3,759.9	3,672.1	102.4

TABLE 12.—*Carbon dioxide, water vapor, and methane eliminated per day, during fast*

Animal and period No.	Subperiods, and days of fast	Carbon dioxide eliminated	Water vapor eliminated	Methane eliminated
		Grams	*Grams*	*Grams*
Steer 47, period 11	Subperiod 1	1,259.36	2,681.72	0.68
	Subperiod 2			
	Third day			
	Subperiod 3	1,108.55	1,898.26	.69
	Subperiod 4	1,180.17	2,043.88	
	Fourth day	2,288.72	3,942.14	
	Subperiod 5	1,115.18	1,818.62	.15
	Subperiod 6	1,146.36	1,667.65	.73
	Fifth day	2,261.54	3,486.27	.88
	Subperiod 7	1,120.16	1,329.19	.61
	Subperiod 8	1,125.89	1,471.76	.17
	Sixth day	2,246.05	3,800.95	.78
Steer 36, period 12	Subperiod 1	1,306.31	1,756.92	.37
	Subperiod 2	* 1,262.18	* 1,940.50	1.60
	Fourth day	2,568.49	3,697.42	1.07
	Subperiod 3	1,204.62	1,685.59	1.29
	Subperiod 4	1,192.51	1,550.64	.07
	Fifth day	2,397.13	3,246.23	1.36
	Subperiod 5	1,128.07	1,437.87	1.09
	Subperiod 6	1,159.46	1,376.25	.90
	Sixth day	2,287.53	2,814.12	1.99
	Subperiod 7	* 1,232.61	* 1,457.94	.79
	Subperiod 8	1,129.88	1,256.59	.82
	Seventh day	2,362.49	2,714.53	1.61

* Corrected for man entering chamber

TABLE 13.—*Heat emission and heat production per day, during fast*

Animal and period No.	Sub-period No.	Calorimeter temperature	Water consumption	Heat emission: By radiation and conduction	Heat emission: As latent heat of water vapor	Heat emission: Total	Correction for body gain	Heat production
		°C.	Kgm.	Cals.	Cals.	Cals.	Cals.	Cals.
Steer 47, period 11	1	17.6	24.38	2,581.45	1,574.17	4,155.62	+377.38	4,533.00
	2							
	3	17.3	1.19	2,635.42	1,116.18	3,751.60	−48.06	3,703.54
	4	17.4		2,725.83	1,201.80	3,927.63	−110.21	3,817.42
	5	17.4	1.17	2,614.78	1,069.35	3,684.13	−53.97	3,630.16
	6	17.3		2,824.01	980.58	3,804.59	−69.07	3,734.62
	7	17.4	1.02	2,755.28	899.16	3,654.44	−37.75	3,616.69
	8	17.4		2,877.74	865.39	3,743.13	−58.24	3,684.89
Steer 36, period 12	1	17.4	8.22	3,182.83	1,033.07	4,215.90	+99.71	4,315.61
	2	17.4		2,992.12	1,141.01	4,133.13	−56.50	4,076.63
	3	17.2	3.04	3,074.28	997.01	4,071.29	−78.20	3,993.09
	4	17.1		3,107.02	911.78	4,018.80	−137.43	3,881.37
	5	17.1	1.79	2,924.43	845.47	3,769.90	−38.18	3,731.72
	6	17.2		3,057.85	809.24	3,867.09	−85.44	3,781.65
	7	17.3	4.94	3,212.71	857.27	4,069.98	+1.34	4,071.32
	8	17.3		3,007.96	738.87	3,746.83	−74.76	3,672.07

TABLE 14.—*Heat production of fasting steers, corrected to the standard day*

Animal and period No. and days of fast	Weight of animal	Time spent standing	Difference from standard of time spent standing	Correction ᵃ	Heat production, uncorrected	Heat production, corrected to standard day
Steer 47, period 11:	Kgm.	Hours	Hours	Cals.	Cals.	Cals.
Fourth day	478.6	6.77	5.23	+378.13	7,520.96	7,899.09
Fifth day	478.6	6.10	5.90	+426.57	7,364.78	7,791.35
Sixth day	478.6	6.77	5.23	+378.13	7,301.58	7,679.71
Average						7,790.01
Steer 36, period 12:						
Fourth day	467.8	10.57	1.43	+101.10	8,392.24	8,493.34
Fifth day	467.8	6.65	5.35	+378.25	7,874.46	8,252.71
Sixth day	467.8	4.90	7.10	+501.97	7,513.37	8,015.34
Seventh day	467.8	9.60	2.40	+169.68	7,743.39	7,913.07
Average of last three days						8,060.37

ᵃ Correction for steer No. 47, 72.3 Cals. per hour; for steer No. 36, 70.7 Cals. per hour.

TABLE 15.—*Contents of alimentary tract of steers after fast*

Animal No.	Contents of—Paunch and reticulum	Omasum	Abomasum	Small intestine	Large intestine	Total, alimentary tract
	Kilograms dry matter					
Steer 47, after 6 days' fast	0.894	0.175	0.229	0.191	0.248	1.737
Steer 36, after 7 days' fast	.517	.281	.089	.151	.089	1.127
	Kilograms fresh substance					
Steer 47, after 6 days' fast	12.100	1.045	2.041	2.392	2.184	19.762
Steer 36, after 7 days' fast	11.285	1.665	1.227	3.015	1.008	18.200

The results of the study of the fasting katabolism are given in Tables 8 to 15, this study, including analysis of the air as it entered and as it left the calorimeter chamber (Tables 8 and 9), determinations of the urinary nitrogen (Table 10), comparisons of the heat production computed from the respiratory quotient with the directly observed heat production (Table 11), data for the carbon dioxide, water vapor, and methane eliminated per day (Table 12), for the heat emission and the heat production per day (Table 13), and for the heat production computed to the standard day (Table 14); also (Table 15) weights of the contents of the alimentary tract after fast.

The measurement of the fasting katabolism (Table 13) began on the third day of fast with steer No. 47, and on the fourth day with steer No. 36.

The third day of fast with steer No. 47, however (subperiods 1 and 2), proved to be too soon after the preparatory treatment for the satisfactory handling of the animal in the calorimeter, and the data for this day were incomplete; and with steer No. 36 the heat production on the fourth day of fast (subperiods 1 and 2) was considerably higher than on the three following days; also the nonprotein respiratory quotients for the two subperiods of this day (0.807 and 0.780; see Table 11) show that a true state of fast had not been reached. The data used, as measures of the fasting katabolism, therefore, were, in the case of steer No. 47, from the fourth, fifth, and sixth days of fast, and with steer No. 36, from the fifth, sixth, and seventh days of fast.

The standardization of procedures in this matter of treatment preliminary to the measurement of the fasting katabolism has not yet been established in all details.

There has been a suggestion, in the course of the heat production on the successive days in the calorimeter during fast, that even after the usual 14-hour preliminary interval another day must elapse before the animal is completely adjusted and resigned to the experimental program; and it is quite conceivable that a longer period of adjustment is necessary with the fasting animal than with the animal which receives feed.

In addition to the course of the heat production during fast, we have, as bases for judgment as to when the animal is in a true post-resorptive state, the conditions as to the gaseous metabolism, and the content of feed residues in the alimentary tract.

The conditions of especial interest with reference to the gaseous metabolism are as relating to the nonprotein respiratory quotient, and to the methane elimination, the former as an indication of the time of exhaustion of the supply of nutriment from the alimentary tract, and also of the status of the glycogen reserve—the time, therefore, at which the animal becomes dependent exclusively on body fat and protein as sources of energy; and the latter—the methane elimination—as an indication of the time at which the fermentation of feed residues in the alimentary tract, with resultant liberation of heat and assimilable nutriment, diminishes to become a negligible factor.

The conditions necessary for the determination of the respiratory quotient were not quite ideal, in that it was necessary to collect the air sample for oxygen estimation over water; but results of significance were obtained.

Carbon dioxide was determined gravimetrically by absorption from measured, continuous aliquots of both ingoing and outcoming air; and oxygen was determined, by means of the Sondén apparatus, in a large, continuous, 12-hour aspirator sample.

The determinations of oxygen in the ingoing air (Table 8) were made in samples of the outside air taken three times per 12-hour subperiod, and are nearly enough in agreement with the known content of oxygen in out-of-door air to show that satisfactory conditions of analysis prevailed.

Also the heat production as computed by the use of respiratory quotients, derived from the analytical data in Tables 8 and 9, shows by comparison with the directly observed heat production, as in Table 11, that the respiratory quotients are essentially correct.

An examination of these nonprotein respiratory quotients indicates that with steer No. 47 a state of true fast existed from the first day of measurement (the third day of fast); but with steer No. 36 the respiratory quotients indicated some oxidation of carbohydrate until the second subperiod (subperiod 4) of the fifth day of fast. It seems possible that there is an appreciable measure of individuality in cattle as to the duration of time necessary to the exhaustion of glycogen reserves.

The high respiratory quotients with steer No. 47 in subperiods 5 and 6, and with steer No. 36 in subperiods 6 and 7, in both cases after the respiratory quotient had been down to a level representative of true fast, may be the result of some imperfection of technic.

The number of grams of carbon dioxide and methane, of carbon in each of these, and of water vapor, given off by the steers in each subperiod during fast, are given in Table 12, these data being used in the computation of the heat emission and heat production, as set forth in Table 13.

The figures representing methane (Table 12) are of especial interest, since from the beginning of the observations during fast (on the third and the fourth days of fast, respectively, with the two steers) the methane produced weighed but a fraction of a gram per 12-hour subperiod—an exceedingly small quantity, in relation to the size of the animal and to the normal methane production during feeding (see Table 5: 162.5 to 183.2 gm. per 24 hours, during periods 1 and 2).

All considered, it seems quite feasible to get a steer into a state of essentially complete fast; but more evidence than is now at hand will be necessary to the final standardization of the method of doing so.

The heat emission by radiation and conduction, and as latent heat of water vapor, the correction for body loss (or gain), and final value for heat production comprise Table 13.

An interesting situation with respect to the method of heat outgo during the progress of fast is revealed by the data for heat emission by radiation and conduction as compared with the parallel figures for latent heat of water vapor. The former remains at about the same level, from subperiod to subperiod, while the latter decreases materially, in 10 among 13 consecutive pairs of subperiods.

The method of experimentation was not such as definitely to reveal the reason for this decrease in latent heat of water vapor, but possible decrease of respiration and of peripheral circulation

and the observed decrease of water intake are suggested as causative factors.

Such a tendency toward a regular decrease in latent heat of water vapor as characterized these fasting periods is not apparent in some of the previous fasting experiments of this institute.

The prominent decrease of water intake during fast was probably due to decreased need for water, perhaps to an increased sensitiveness to heat loss, as through the drinking of water at a temperature lower than that of the body.

In the light of a recent study at this institute (*7*) it is the opinion of the writers that the steers were at no time subjected to subcritical temperatures.

About an hour and a half after the termination of the fasting calorimetric periods the steers were killed in order to permit the quantitative accounting for the feed residues in the alimentary tract, as indicating the validity of conditions for the fasting heat measurement, and to make possible the direct determination of the area of the hide as a measure of the surface area of the animal.

The dry matter of the feed residues found in the alimentary tract of steer No. 47 was 1.737 kgm., and of steer No. 36, 1.127 kgm. (Table 15). Of these quantities, however, only 29 and 38 per cent, respectively, were present in the true stomach (abomasum) and intestines. The remaining 71 and 62 per cent, respectively, were found in the paunch, reticulum, and omasum, from which there is little or no absorption of nutrients. The feed present in these portions of the alimentary tract, therefore, was negligible from the point of view of the present interest; that is, as available nutriment, or as contemporary feed residues.

These observations show that these steers were unable completely to empty the paunch, even in the course of a seven-day fast, but that conditions within the alimentary tract, at the termination of the fasting heat measurement, represented practically complete fast.

DETERMINATION OF THE SURFACE AREA OF THE EXPERIMENTAL SUBJECTS

The most significant conventional base value thus far proposed, to which to relate the maintenance requirement of cattle, is the surface area; but this value is not easily determined.

TABLE 16.—*Measurements of surface area of steers after fast*

Steer No.	Measured area of hide	Computed by Moulton's formula	Computed by Hogan's formula
	Sq. meters	*Sq. meters*	*Sq. meters*
47	(a) 4.931 (b) 4.882	5.301	5.009
36	4.950	5.305	4.763

In Table 16 are estimations of the surface area of the two steers, determined (1) by the measurement of the removed hide; (2) by Moulton's formula (*21*), $A = 0.1186\ W^{5/8}$, in which *A* is the surface

area in square meters, and W the warm empty weight in kilograms; and by Hogan's formula (*15*), $S = W^{0.4} \times L^{0.8} \times K$, in which S is the surface area in square centimeters, W is the weight in kilograms, L is the length of the body in centimeters, and K is the constant, 217, for cattle.

The satisfactory use of Moulton's formula would require the further standardization of conditions and procedure in computing the empty weight. In the present use of this formula the empty weight was computed from the gross live weight during maintenance feeding, by multiplying by 0.9, as proposed by Moulton.

Direct measurements of the surface area of cattle have been made by Brody (*4*), by means of a so-called surface integrator; but it seemed impracticable thus to measure the surface area of the steers used in these experiments on account of their thick, wooly coats and their nervous disposition.

The most satisfactory method for determining the surface area of cattle seemed to the authors to be the measurement of the area of the removed hide. Accordingly, the freshly removed hide of each of the two steers used in these experiments was smoothed out, without stretching, on a concrete floor, and the outline drawn with chalk.

The main part of the area so inclosed was ruled into rectangles, and measured; and tracings were made, on paper, of the irregular areas at the edges.

The areas of these tracings were then measured with a planimeter; and, for comparison, their area was also computed from the weight of the pieces inclosed by the tracings in relation to that of a measured square of the same paper.

These two methods of measurement of the area of the tracings gave results agreeing satisfactorily; and the second was found much the easier to employ. The two values for the surface area of steer No. 47, designated (a) and (b), were both obtained by this second method of measurement.

This direct determination of the area of the removed hide, therefore, has been adopted as the most accurate and practicable measure of the surface area of cattle—for use in experiments in which the life of the subjects may be sacrificed.

The surface area of the steers, as thus determined by the measurement of the hides after fast, was made the basis of a computation of the surface area during the preceding maintenance period, by the following procedure:

First, the empty body weight during maintenance was computed by adding to the empty body weight after fast, as directly determined by slaughter test, the loss in body weight during fast, as computed from the loss in carbon and nitrogen.

Then the surface area of the steers at the maintenance level was computed by increasing the surface area (hide measurement) during fast in proportion as the five-eighths power of the computed body weight during maintenance was greater than the five-eighths power of the observed empty body weight after fast, this computation being based on Moulton's formula (*21*) for determining the surface area from the empty body weight.

The maintenance requirement of net energy was finally computed by multiplying *the number of square meters of surface area during*

maintenance by the number of Calories of heat production per square meter of body surface as determined during fast. The data involved in these computations are given in Table 17.

TABLE 17.—*Derivation of values for surface area and maintenance requirement of net energy during the maintenance period preceding fast*

Animal No.	Surface area during fast	Fasting katabolism		Empty weight of fasting animals	Loss of body tissue after maintenance until end of fast	Computed empty weight in maintenance period	Computed surface area in maintenance period	Maintenance requirement of net energy per head
		Total	Per square meter of body surface					
	Sq. meters	*Cals.*	*Cals.*	*Kgm.*	*Kgm.*	*Kgm.*	*Sq. meters*	*Cals.*
47	4.906	7,790	1,588	444.8	13.8	458.6	5.000	7,940
36	4.950	8,060	1,628	436.5	16.8	453.3	5.068	8,251

FINAL COMPUTATIONS

The results of the final computations based on the experimental data, for the purpose of bringing out the facts as to the utilization of the energy of the feed, comprise Tables 18 to 23. The objects of these computations and the methods of making them will be briefly explained in the following paragraphs, and the results will be discussed later, under several appropriate headings.

TABLE 18.—*Heat production corrected to a basis of uniform live weight and maintenance requirement*

Animal No. and plane of nutrition	Period No.	Average live weight	Fasting katabolism (maintenance requirement)	Correction to be applied to heat production	Heat production: Uncorrected for live weight		Heat production: Corrected to uniform live weight	
					Observed	Computed	Observed	Computed
Steer No. 47:		*Kgm.*	*Cals.*[a]	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Fast	11	478.6	7,790		7,790		7,790	
Half less than maintenance	5	474.8	7,749	+41	8,077	8,218	8,118	8,259
Maintenance (mixed ration)	7	484.8	7,857	−67	9,756	9,983	9,689	9,916
Half more than maintenance	3	494.6	7,963	−173	11,999	12,354	11,826	12,181
Twice maintenance	1	486.2	7,872	−82		13,793		13,711
Maintenance (hay only)	9		[b]7,940	−150	11,609	11,525	11,459	11,375
Steer No. 36:								
Fast	12	467.8	8,060		8,060		8,060	
Half less than maintenance	6	471.2	8,099	−39	8,377	8,569	8,338	8,530
Maintenance (mixed ration)	8	481.2	8,213	−153	10,022	10,212	9,869	10,059
Half more than maintenance	4	490.2	8,315	−255	11,973	12,230	11,718	11,975
Twice maintenance	2	482.9	8,232	−172		13,786		13,614
Maintenance (hay only)	10		8,251	−191	11,575	11,950	11,384	11,759

[a] Computed to correspond to the average live weight of fasting in proportion to the two-thirds power of the live weights.

[b] Correction based on estimated loss of body tissue.

TABLE 19.—*Heat production, corrected for differences in live weight, and heat increments derived by comparison of the heat production in the periods of feeding with that of fast*

Animal and period No.	Heat production: Observed	Heat production: Computed	Heat production: Average	Fasting katabolism	Heat increments: Total	Heat increments: Per kilogram of dry matter
Steer No. 47:	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Period 1	------	13,711	13,711	7,790	5,921	802
Period 3	11,826	12,181	12,004	7,790	4,214	750
Period 5	8,118	8,259	8,189	7,790	399	214
Period 7	9,689	9,916	9,803	7,790	2,013	531
Period 9	11,459	11,375	11,417	7,790	3,627	628
Steer No. 36:						
Period 2	------	13,614	13,614	8,060	5,554	789
Period 4	11,718	11,975	11,847	8,060	3,787	707
Period 6	8,338	8,530	8,434	8,060	374	198
Period 8	9,869	10,059	9,964	8,060	1,904	506
Period 10	11,384	11,759	11,572	8,060	3,512	609

TABLE 20.—*Partition of energy of feed*

Animal and period No.	Dry matter of feed mixture	Energy per kilogram of dry matter: Gross	Digestible	Metabolizable	Total net [a]	Feces	Heat increment	Methane	Urine
Steer 47:	*Kgm.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.* [b]	*Cals.*	*Cals.*
Period 1	7.384	4,519	3,145	2,644	1,842	1,374	802	331	170
Period 3	5.617	4,519	3,283	2,762	2,012	1,236	750	335	186
Period 7	3.790	4,518	3,358	2,772	2,241	1,161	531	382	203
Period 5	1.863	4,518	3,304	2,628	2,414	1,214	214	444	232
Steer 36:									
Period 2	7.037	4,519	3,127	2,646	1,857	1,392	789	308	173
Period 4	5.358	4,519	3,273	2,734	2,027	1,246	707	344	195
Period 8	3.762	4,518	3,331	2,724	2,218	1,187	506	394	213
Period 6	1.885	4,518	3,284	2,507	2,309	1,234	198	441	246

[a] Mixed values derived by the use of heat increments computed as in Table 19. Net-energy values for maintenance and for production are given in Tables 21 and 22.
[b] Based on the average of the observed and computed heat production corrected for differences in live weight, and computed as in Table 19.

TABLE 21.—*Net-energy values for maintenance*

Period No.	Steer No.	Dry matter of feed eaten: Alfalfa hay	Dry matter of feed eaten: Corn meal	Dry matter of feed eaten: Total	Total heat production	Fasting katabolism	Heat increments: Total	Heat increments: Per kilogram of dry matter	Metabolizable energy per kilogram of dry matter	Net energy per kilogram of dry matter (for maintenance)	Utilization of metabolizable energy (for maintenance)
		Kgm.	*Kgm.*	*Kgm.*	*Cals.* [a]	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Per cent*
9	47	5.771	------	5.771	11,417	7,790	3,627	628	2,013	1,385	68.60
10	36	5.763	------	5.763	11,572	8,060	3,512	609	2,004	1,395	69.61
7	47	1.911	1.879	3.790	9,803	7,790	2,013	531	2,772	2,241	80.84
8	36	1.902	1.860	3.762	9,964	8,060	1,904	506	2,724	2,218	81.42
Computed values of corn meal	47	------	------	------	------	------	------	433	3,544	3,111	87.78
Do.	36	------	------	------	------	------	------	401	3,460	3,059	88.41

[a] Average of the observed and computed heat production corrected for differences in live weight.

TABLE 22.—*Net-energy values of ration, or component, for body increase*

Steer No.	Periods compared	Ration or component	Planes of nutrition compared	Heat increment per kilogram of dry matter	Metabolizable energy per kilogram of dry matter in supermaintenance periods	Net energy per kilogram of dry matter (for body increase)	Utilization of metabolizable energy (for body increase)
		Kgm.		*Cals.*	*Cals.*	*Cals.*	*Per cent*
47	1 and 7	Hay and meal	Twice maintenance and maintenance.	1,087	2,644	1,557	58.89
36	2 and 8	do	do	1,115	2,646	1,531	57.86
47	3 and 7	do	Half more than maintenance and maintenance.	1,205	2,762	1,557	56.37
36	4 and 8	do	do	1,184	2,734	1,550	56.69
47	1 and 7	Alfalfa	Twice maintenance and maintenance.	[a] 1,286	1,920	634	33.02
36	2 and 8	do	do	[a] 1,342	1,947	605	31.07
47	1 and 7	Corn meal	do	[a] 886	3,380	2,494	73.79
36	2 and 8	do	do	[a] 884	3,361	2,477	73.70
47	3 and 7	Alfalfa	Half more than maintenance and maintenance.	[a] 1,425	2,006	581	28.96
36	4 and 8	do	do	[a] 1,425	2,011	586	29.14
47	3 and 7	Corn meal	do	[a] 993	3,531	2,548	72.16
36	4 and 8	do	do	[a] 936	3,472	2,535	72.99

[a] See p. 280 for method of derivation.

TABLE 23.—*Heat increments as computed from differences in the heat production of consecutive planes of nutrition*

Steer No.	Periods compared	Planes of nutrition compared	Heat increment per kilogram of dry matter
			Cals.
47	11 and 5	Fast and half less than maintenance	214
	5 and 7	Half less than maintenance and maintenance	838
	7 and 3	Maintenance and half more than maintenance	1,205
	3 and 1	Half more than maintenance and twice maintenance	966
36	12 and 6	Fast and half less than maintenance	198
	6 and 8	Half less than maintenance and maintenance	815
	8 and 4	Maintenance and half more than maintenance	1,184
	4 and 2	Half more than maintenance and twice maintenance	1,049

The object of the computations represented by Table 18 is to derive values for the heat production of the steers, in different experimental periods and on different planes of nutrition, reflecting the influence of the feed, and being free from the influence of the different live weights of the animals in the several experimental periods.

To this end the maintenance requirement of net energy, in the several periods, was computed from the fasting heat production by modifying this value in proportion to the two-thirds power of the average live weights; and the heat production in the several periods was corrected to a basis of uniform live weight (and maintenance requirement) by adding or subtracting, according to the nature of the correction, the difference between the heat production as observed during fast and the corresponding maintenance quota of net energy, computed as above, for the several feeding periods.

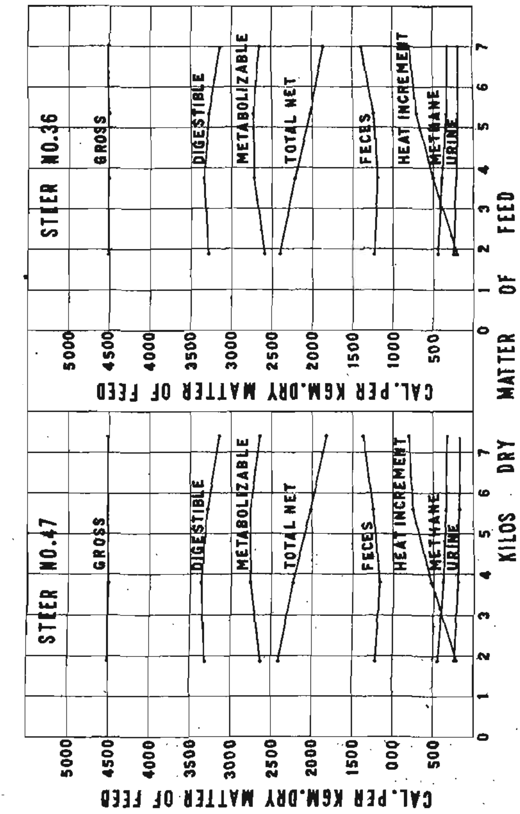

FIG. 1.—Partition of feed energy as influenced by the plane of nutrition of cattle

The uncorrected heat production, as directly observed and as computed by the balance method, and these same values corrected, as above indicated, to a basis of uniform live weight and maintenance requirement, are given in the four columns at the right in Table 18.

In Table 19 the computation is carried a step further, to the total heat increments (energy expenditure of feed utilization) per kilogram of dry matter of feed, in the several periods. These values were obtained by subtracting the heat production of fast from the average of the observed and the computed heat production in each period, corrected, as shown in Table 18, to the basis of uniform live weight and maintenance quota, and dividing by the number of kilograms of dry matter of feed. It should be noted that heat-increment values [4] computed in this manner cover maintenance and production together.

The partition of the gross energy of the ration of corn meal and alfalfa hay, according to the designations—digestible, metabolizable, urine, feces, methane, total heat increment, and total net—as affected by the plane of nutrition, is shown in Table 20 and in Figure 1.

The total net-energy values in this table are for maintenance and body increase together. In view of the fact, therefore, that food energy is used for maintenance and for body increase at different rates of economy, these figures for net energy are mixed values, applying only to the particular planes of nutrition at which the determinations were made and having no general or standard significance.

The difference between these total net-energy values and the separate values for maintenance and for body increase, to be discussed, should be clearly appreciated.

In Table 21 are (1) the net-energy values, for maintenance of the alfalfa hay, of the mixed ration, and of the corn meal, (2) the percentage of utilization of the metabolizable energy of each of the foregoing, and (3) the data leading directly to these values. The net-energy values of the mixed rations for maintenance, with the corresponding heat-increment values, are represented as "Net 1," and "Heat Incr. 1," in Figure 2.

The computation of the net-energy values for maintenance was made by the usual method—the values for the mixed ration and for the alfalfa hay being determined directly by subtracting from the metabolizable-energy value the heat-increment value obtained by comparison of the heat production of maintenance with that of fast; and the value for corn meal being computed by difference, from the net-energy values for the mixed ration and for the hay, by assuming that the hay in the mixed ration has the same net-energy value as that determined for the hay when fed alone.

The utilization of metabolizable energy, as expressed in the last column of Table 21, was computed as the percentage of the metabolizable which is net.

The net-energy values of the mixed rations, for body increase, as recorded in Table 22, were computed by the same general procedure as for maintenance, but with the difference that the heat-increment values were computed from comparisons of the heat production at the higher levels of nutrition with the heat production of maintenance

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[4] The terms "metabolizable energy," "heat increment," and "net energy," according to the usage of this institute, are without definite signification as to quantities of energy of these descriptions; but "metabolizable-energy values," "heat-increment values," and "net-energy values" are the quantities of each per kilogram of dry matter of feed.

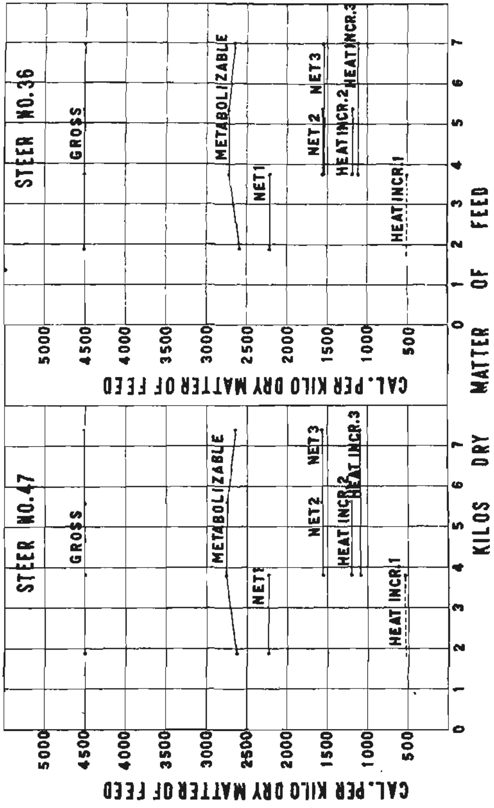

FIG. 2.—Net energy of rations resolved into components for maintenance and for body increase

instead of with that of fast; and the computation of the net-energy values of the individual feeds for body increase was performed by a new procedure, which has been mentioned (p. 257), depending on the assumption that the heat increment and the metabolizable energy of the individual feeds are affected by the plane of nutrition in the same ways and to the same extent as are the same functions of the mixed ration. The net-energy values of the mixed rations for body increase, with the corresponding heat-increment values, are represented, with similar values for maintenance, in Figure 2.

HEAT-INCREMENT, METABOLIZABLE-ENERGY, AND NET-ENERGY VALUES

The heat-increment values for alfalfa hay (Table 22, first column of figures) are decidedly higher than are those relating to corn meal alone, or to the mixed ration, though the metabolizable-energy value of the alfalfa hay is much lower than that of the mixed ration or of the corn meal.

These high heat-increment values of alfalfa hay are due, in large part at least, to the fibrous nature of this feed; but they also direct attention to the effect of its protein constituents in stimulating heat production.

The percentages of utilization of metabolizable energy, as given in the last column, agree almost perfectly for the two steers, and differ in accord with the kind of feed in an apparently consistent manner.

The net energy of the mixed ration and of the individual feeds, for body increase, are given in the third column of figures.

The uppermost four values apply to the mixed ration, the first pair to the interval between maintenance and twice the maintenance requirement, and the second pair to the interval between maintenance and half more than maintenance.

The determinations with steer No. 47 at these two planes of nutrition gave exactly the same value, 1,557 Calories, while the determinations with steer No. 36 also agreed well, being 1,531 and 1,550 Calories, respectively.

There is a distinct difference between the heat-increment values of the ration at these two levels of production, those values involving the larger quantity of feed being the lower. Owing, however, to the fact that the metabolizable-energy values of the ration in the periods of highest feed intake are lower than in the periods of feeding half more than the maintenance requirement, by quantities almost the same as the differences in heat increments, the two sets of net-energy values are almost identical.

The net-energy values of the mixed ration at the two higher planes of nutrition, therefore, seem to be alike.

The second four values represent the plane of nutrition from maintenance to twice the maintenance requirement, the first pair applying to alfalfa hay and the second pair to corn meal; and the third four values represent the plane of nutrition from maintenance to half more than maintenance, the first pair applying to alfalfa and the second to corn meal.

At the higher plane of production (maintenance to twice maintenance) the alfalfa hay has a value somewhat higher than at the lower plane (maintenance to half more than maintenance), which, by the

"difference method" of computation, gives to the corn meal at the higher plane a somewhat lower value than at the lower plane.

These differences, however, may represent only experimental error, since the values for the individual feeds are derived by a computation involving not only the difference method, as stated, but also extensive modification of the heat-increment and metabolizable-energy values, as directly determined.

The agreement of the percentages of utilization of metabolizable energy of the mixed ration at the two planes of nutrition is good; and the data for the alfalfa hay as compared with those for the corn meal, at the two planes of nutrition, call attention to the fact that the net-energy value of the hay is lower than that of the grain both because the metabolizable energy of the hay is lower and because its heat increment is higher than that of the grain.

It must be understood that the net-energy values for body increase, which have been discussed, apply not to the ration as a whole but only to that portion of the ration which is in excess of the maintenance requirement.

HEAT PRODUCTION AS AFFECTED BY THE PLANE OF NUTRITION

The heat production as related to the plane of nutrition is exhibited in Figure 3. The data for heat are the averages of the observed and the computed values, corrected to the standard day, and also corrected to correspond to a uniform live weight and maintenance requirement (Table 10); and the data representing the kilograms of dry matter of feed eaten per day may be found in Table 5, or in Table 20.

The order in which the five planes of nutrition were studied is shown by the numerals at the points of observation in the graph, this order having been determined in consideration of a possible carrying over of an influence in relation to the heat production from one period and plane of nutrition to a following period and different plane of nutrition.

Accordingly, the animals were first given the largest amount of feed; that is, twice the maintenance requirement, followed in order by half more than maintenance, half less than maintenance, maintenance, and fast.

In this study it was considered especially important to reach a correct value for fasting, an object rendered difficult by the fact that the heat production of fast, however determined, is not an absolute value, but is somewhat affected by the conditions of determination, especially in regard to the duration of fast and to the previous plane of nutrition. The authors have proceeded, therefore, with the understanding that the methods of determination of the fasting katabolism, as a measure of the maintenance requirement of net energy, must be rigidly standardized; and the sequence of treatments was so arranged, as a routine procedure, that fasting periods should always follow periods at the maintenance level of nutrition. Careful attention has been given the control of this measurement, and the authors feel that the maximum aggregate error of work affecting the values used in this study can not be of such magnitude as would materially alter the curve of heat production in relation to plane of nutrition.

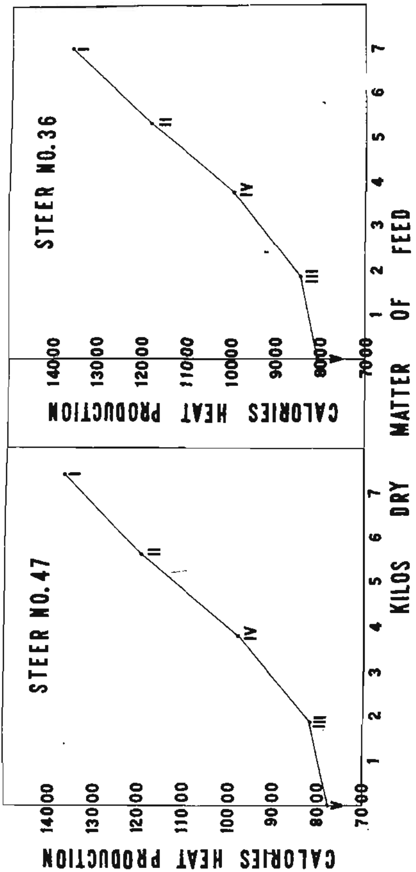

FIG. 3.—Heat production as affected by the plane of nutrition of cattle

A study of Figure 3 shows, at once, a remarkable concordance of the curves representing the heat production of the two steers at the several planes of nutrition. This agreement indicates that the experimental routine was well in hand, and especially that the validity of the results, for comparative purposes, was not seriously vitiated by effects of any uncontrolled and unrecorded physical activity of the experimental subjects.

The heat production increased but little from fast (V) to the plane of half of the maintenance requirement (III), and increased much more rapidly from the latter plane of nutrition to the maintenance level (IV); but the entire rise in metabolism from the fasting to the maintenance level was at a distinctly lower rate than was the fairly uniform increase from the level of maintenance (IV) to that of twice maintenance (I).

Several factors appear to contribute to the determination of the character of this curve representing the relation of the heat production to the plane of nutrition.

In harmony with the point of view of this paper the maintenance requirement of net energy is a constant measured by the heat production of fast, which makes no contribution to the curve of heat production except as its amount, or height, fixes the lower point of the curve.

In this light the curve representing the heat production at increasing rates of food consumption may be explained as resulting from the following influences: (1) The stimulation of the heat production through the specific dynamic effect of protein and the so-called "metabolism of plethora" (of fat and carbohydrate), as Lusk uses this term; (2) the energy expenditure of synthesis of body nutrients above maintenance (fat, from carbohydrate); and (3) the decreased metabolizability of the food at the higher planes of nutrition.

The slight divergence of the curve toward the horizontal at the highest as compared with the next lower plane of nutrition is apparently due in part at least to this last condition; that is, to the fact that the amounts of food available for the use of the animal are not quite proportional to the amounts of food consumed.

From another point of view, on hypothetical grounds not in harmony with the fundamental conception of the observed heat production of fast as the measure of the maintenance quota of net energy, an important factor in the determination of this curve of heat production would be an assumed specific dynamic effect of body nutrients katabolized below maintenance.

Thus, differences in the specific dynamic effect of circulating metabolites, due to the decreasing amount of body nutrients and increasing amount of food nutrients katabolized between fast and maintenance, and to the changing proportions of fat, protein, and carbohydrate in the body nutrients katabolized between fast and maintenance, would contribute prominently to the composition of this curve.

In accord with this hypothesis the maintenance quota of net energy would be conceived of as the heat production during a status of fast in which the energy requirement of the animal would be rendered available without waste of heat, that is, without energy expense of utilization; this heat production, therefore, being a true measure of

the energy requirement. This hypothetical minimum maintenance requirement, then, would be a lower value than the actual fasting katabolism by an amount assumed to represent an energy expense of utilization, or a specific dynamic effect, of body nutrients oxidized during fast.

The hypothetical minimum maintenance requirement of net energy above suggested is not now a practicable or a desirable base value for use in studies of the energy of feeds, however, (1) since it can not be measured directly; (2) since no method has been devised for computing it with satisfactory invariability; and (3) since this conception would necessitate the abandonment of the directly determined and universally accepted measure of the maintenance quota in favor of a computed datum the physiological significance of which has not been established.

Also, if there were an opportunity to choose between these two measures of net-energy requirement of maintenance, it would not be quite as between a correct and an incorrect value; because although the lower figure would give a lower net-energy value, and the higher, a higher net-energy value, of the feed, the quantity of feed required for maintenance would be reckoned as the same in either case.

It may be helpful in appreciating the significance of the two standards of measurement of the maintenance requirement of net energy in relation to the curve of heat production, to consider that they differ as though between regarding this curve from its upper end, from which point of view the striking fact is the increased heat production in relation to feed eaten, below maintenance; and from its lower end, from which point of view there is a marked increase in heat production in relation to feed eaten, above maintenance.

The final decision between these two points of view, that is, as to whether the curvature of the line representing the heat production between the levels of fast and maintenance expresses a factor of waste in the heat produced from the body substance katabolized, or a variation in the energy cost of utilization of food, must await the quantitative analysis of the heat production of fast, that is, the measurement of the factors contributing thereto.

Whatever the point of view from which the curve of heat production is regarded, and whatever the value employed as the maintenance requirement of net energy, it is well to realize that the energy cost of utilization of food for maintenance is measured as the difference between the maintenance requirement of net energy (however measured) and the heat production at the maintenance level; the net-energy value of the food, for maintenance, and the relative efficiency of utilization of food for maintenance as compared with body increase, therefore, are directly affected by the magnitude of the figure (directly observed fasting heat production, or lower, hypothetical, minimum heat production) accepted as the measure of the maintenance quota of net energy.

During fast, body tissue is used as a source of energy for the vital activities of the animal, and, according to present usages, the maintenance requirement of net energy is measured by the metabolizable energy of the body tissue katabolized.

If, however, a sufficient quantity of feed is given to prevent any loss of body tissue, the feed instead of the body tissue is used as a

source of energy for the vital activities of the animal, that is, for maintenance; and the net-energy value of the feed, the determination of which present usage involves the comparison of the heat production of fast and maintenance, is its value for the prevention of body loss, that is, for maintenance.

Above the maintenance level there is no replacement of body nutrients by feed nutrients, as there is below maintenance, and the amount by which the heat production is greater than that at maintenance is a direct measure of the cost of utilization of feed energy for the production of body increase.

The curves, as well as the heat-increment values in Tables 21 and 22, show a distinct difference between the terms of the two uses of feed energy, and that it is utilized more efficiently to prevent loss than to produce increase of body tissue.

It is impossible to assume, therefore, as was done in the publications of this institute up to 1925, except on the basis of the hypothesis on page 285, which is not definitely advocated, that the rate of utilization of feed energy is the same above as it is below the maintenance level of nutrition.

The most practicable method of recognition of these differences is through the determination of separate net-energy values of feeds for maintenance and for body increase, as recorded in Tables 21 and 22, and as discussed in a later section of this paper.

In this connection mention should be made of the rate of utilization of feed energy for milk production, which was shown in a recent paper (*9*) from this institute to be essentially the same as that at which it is utilized for maintenance. This may be understood as consistent if the milk is considered as potential but unorganized body substance, and the nutritive requirement of milk production as an increase in the maintenance requirement of the body, since during milk production and liberal feeding the body itself remains approximately in energy equilibrium.

A consequence of the facts as exhibited with reference to the relationship between the heat production and the plane of nutrition, between fast and maintenance, which should be considered in the determination of the specific dynamic effects of foods, or of nutrient compounds, at planes of nutrition between these levels, in comparison with fast, is that such dynamic effects must differ prominently with the planes of nutrition at which the observations of heat production are made. In this light it appears that comparable determinations of specific dynamic effects of nutrients can be made only at the same plane of nutrition.

Figure 4 is presented in an effort to make clearer the relations between the heat production, the gross energy, the metabolizable energy, the maintenance quota, the heat increment, the body increase (above maintenance), and the body loss (below maintenance) as affected by the plane of nutrition. For this purpose the actual results obtained with steer No. 47 have been plotted.

The area *BAK* covers the gross energy of the rations. The very slight departures of the line *BA* from a straight line are the result of slight differences in the composition of the same kinds of feed used in the several experimental periods.

The area *BAC* covers the energy of the excreta; and *CAK*, the metabolizable energy of the rations.

The point *J* represents the heat production of fast, which is the measure of the maintenance requirement of net energy; and the line *JE* expresses the assumption that the maintenance requirement of net energy is a constant at all planes of nutrition.

The line *JD* (heat) crosses the line *AC* (metabolizable energy) at *F*, the point of energy equilibrium.

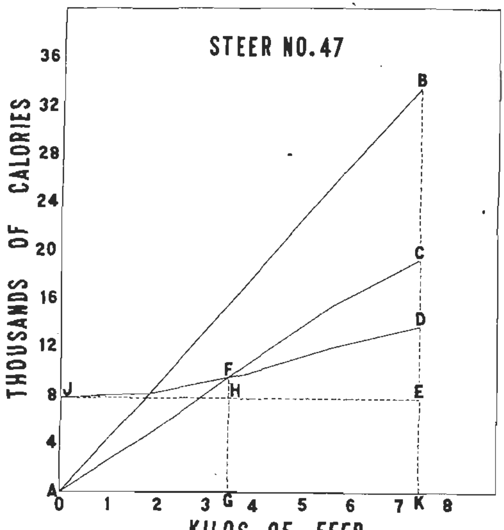

FIG. 4.—Relations of metabolizable energy, heat production, and maintenance quota of net energy to the gross energy of the feed

The area *DJE*, the difference between the heat production and the maintenance requirement of net energy, represents the heat increment or energy expenditure of feed utilization.

Since the heat increment is a part of the metabolizable energy it would be represented in this natural relation with the line *JD* in the line *AC*, the point *E* falling near the point D.

The area *FJH* expresses the heat increment below maintenance; and *DFHE*, the heat increment above maintenance.

The area *FAG* represents the metabolizable energy of the feed. and also the heat produced from the feed, below maintenance; *JFA*, the heat produced at the expense of body substance, below

maintenance; *CFD*, the energy of the body increase, above maintenance; while the net energy of the rations would be represented as the difference between the metabolizable energy, *CAK*, and the heat increment, *DJE*.

As results of direct experimental observations the metabolizable energy, the heat increment, the body gain, and the body loss are all curvilinear functions.

It will be impossible to explain these observations in fundamental ways until the quantitative analysis of the fasting katabolism, and of the heat increment as affected by the plane of nutrition, have been accomplished, and until the reasons for the relations of the digestibility and the metabolizability of the food to the plane of nutrition are understood.

DIGESTIBILITY OF THE FEED AND PARTITION OF THE GROSS ENERGY IN RELATION TO THE PLANE OF NUTRITION

In the exhibit of the disposition of the feed energy, in Calories per kilogram of dry matter of the ration of corn meal and alfalfa hay as affected by the plane of nutrition, set forth in Table 20 and in Figure 1, the points of observation were the same as those indicated in Figure 3; and, as in Figure 3, the agreement between the data representing the metabolism of the two animals is so remarkably close that all general conclusions made with reference to one apply to the other.

The gross energy of the feed at the four planes of observation was, of course, the same, as shown by the horizontal line in Figure 1.

The energy of the digestible nutrients rose very slightly from submaintenance to maintenance, with both steers, this increase being due to a greater digestibility of the crude fiber of the ration at the maintenance than at the submaintenance level. (See Table 2.)

The fall in the curve of energy of digestible nutrients, from the maintenance level to half more than maintenance, and a second fall from the level of half more than maintenance to the highest level, was due mainly to decreased digestibility of carbohydrates and protein.

The significance of data representing digestible nutriment of rations for ruminants differs somewhat from that of such data for other animals, since the method used for computing digestibility reckons the methane produced by the characteristic fermentation in the ruminant paunch as digestible, which, of course, it is not—in a physiological sense.

The metabolizable energy is that portion of the feed energy which can be converted by the animal into heat, or into body products other than excreta. It is the gross energy of the feed minus the energy of the urine, feces, and methane. Since, in this study, the methane and urine curves are nearly straight lines, the proportions of the feed energy so represented diminishing slightly with rise in the plane of nutrition, the curve of metabolizable energy closely follows that of digestible energy, the difference between the two diminishing slightly with increase in feed.

The curve representing the energy of the feces is, necessarily, exactly the reverse of that representing the energy of the digestible nutrients.

The potential energy of the methane produced by cattle from various feeds commonly varies from about 6 to about 11 per cent

of the gross energy; and in the experiment here discussed varied from 6.4 to 9.8 per cent of the gross energy. If the heat of the fermentation which liberates the methane is, as reckoned by Krogh (*18*), 50 Calories per gram molecule of CH_4, this would be equal to about one-fourth of the potential energy of the methane, and therefore to 17.5 per cent of the heat increment of the feed between fast and maintenance in this study.

The curve of methane production, indicating a slight decrease per kilogram of feed, with increase in the plane of nutrition, is in harmony with observations on this point at this institute during many years and is probably the result of less protracted stay of the larger than of the smaller intake of feed in the paunch, a decrease in feed being followed by a smaller proportionate decrease in paunch content.

The line representing the energy of the urine is nearly straight and nearly horizontal, and would be expected to conform perfectly to both conditions if the correction of the urinary energy, given in Table 4, were exact. The slight divergence of this curve from the expected straight and horizontal line may be due to inaccuracy of the factor used in making the above-mentioned correction, or to some unknown cause.

Figure 1 shows that the total heat increment per unit of feed (computed in each case with reference to the fasting heat production) rose significantly with rise in the plane of nutrition, but the rate of increase in heat increment was such as to be expressed not by a straight, ascending line, but by a curve diverging downward from such a line. Obviously, these widely differing heat increments suggest a different net-energy value at each plane of nutrition; but a way out of the dilemma thus presented is shown in the discussion of net-energy values for maintenance and for body increase.

Finally, the total net-energy values of the rations decreased with increase in the plane of nutrition as a practically rectilinear function of the quantity of the feed; and, as has been stated in connection with the discussions of the heat increments, these are in a sense mixed values, each having a different significance. Reading from left to right, the first represents the highly efficient utilization of feed energy to prevent body loss at a very low plane of nutrition (half of the maintenance requirement); the second represents the less efficient utilization of feed energy at the plane of energy equilibrium; and the third and fourth represent the same value for net energy for maintenance plus different quantities of net energy for body increase, the latter being used at a lower rate of efficiency.

The net energy of the rations resolved into components for maintenance and for body increase is indicated in Figure 2 and will be discussed in the following section.

SIGNIFICANCE OF THE RESULTS OF THIS STUDY IN RELATION TO THE PROBLEM OF METHODS OF DETERMINATION OF THE NET-ENERGY VALUES OF FEEDS

The influence of the plane of nutrition on the problem of methods of determination of net energy will be considered in relation (1) to mixed rations, (2) to individual feeds, (3) to maintenance, and (4) to production.

It has long been a matter of common knowledge and observation that the digestibility of feeds is affected by their combination in rations in such sense that the digestibility of the components of a ration may be greater, or, in some cases, less, than if the feeds are eaten separately; and the net energy also is directly affected through this influence, since only digested nutrients contribute to the net energy.

In this matter the situation of especial interest is the reciprocal effect of grain and roughage, since the digestibility of the grain by ruminants is always determined by difference between results from mixed grain and hay rations and rations of hay alone, for the reason that, inasmuch as ruminants do not ruminate when fed grain alone, it is considered that grain would not be normally metabolized under such conditions.

In the determination of the digestibility of grain by ruminants, therefore, the entire effect of combining the grain and roughage is assigned to the grain. This is obviously illogical, and the error seems inevitable; but the situation is not so unsatisfactory as it at first appears, because since grain is fed to ruminants only in combination with roughage, the digestibility determined by difference, as above indicated, while perhaps actually in error, does apply to the general conditions under which grain is fed.

This aspect of the problem, therefore, may be dismissed from further consideration for the present; and inasmuch as the energy of the urine and of the methane produced from the mixed ration fed in these experiments is shown in Figure 1 to be in both cases an essentially rectilinear function of the dry matter of the feed, and further since these two factors of energy outgo are not influenced in important ways by the combination of feeds, they likewise require no further consideration in tbis relation.

Regarding the heat increment, however, the problem is complicated by the facts that this is not a rectilinear function of the dry matter of the feed; that we do not know that the heat increment of combined feeds is under all conditions the sum of their separate heat increments; or that the heat increments of all components of a ration are affected by the plane of nutrition in proportion to such effect on the heat increment of the ration as a whole.

In these experiments it so happens that the feces curve, combined with the heat-increment curve, would result in a practically straight line. Then, since the urine and methane curves are nearly straight, the curve representing the combination of all these losses and expenses of feed utilization must be a nearly straight line, as obviously is also the gross energy, and therefore the total net energy, since the net is the difference between the gross energy and the sum of the losses and expenses of utilization.

The net-energy values of the ration for maintenance and for the two rates of supermaintenance feeding, computed by the method given on page 280, and as indicated as Net 1, Net 2, and Net 3, in Figure 2, are, as a result of the method of derivation, necessarily rectilinear functions; also the net-energy value of the ration at the two planes of supermaintenance feeding (Table 22) were, with steer No. 47, identical, and with steer No. 36, essentially alike.

The net-energy values represented in this graph were computed by the subtraction of the corresponding heat-increment values, as there shown, from the metabolizable energy.

In regard to the net energy of the ration as a whole, either for maintenance or production, then, the situation in the light of the results of these experiments seems satisfactory. The evidence at hand does not afford the basis for final judgment, however, as to the practicability of determining net-energy values of individual feeds, particularly at the production levels of nutrition, since, as indicated above, we are in need of further information as to the effects of feed combination on the dynamic potencies of the individual feeds involved, and especially since in the event that the dynamic effect of a ration of hay and grain together should be found not to be the sum of the dynamic effects of these components, and further should it be shown that the influences of the plane of nutrition on the heat increments of each component of a ration is not proportional to such influence on the ration as a whole, such differences would have to be assigned, though improperly, to the grain alone—which might leave the dependent net-energy values of individual feeds quite without distinctive significance.

TABLE 24.—*Distribution of gross energy of feeds*

	Experiment No.	Gross energy of dry matter	Losses of chemical energy in—			Percentage of nutriment that is digestible	Heat increment for maintenance	Net energy for maintenance	Percentage of energy of digestible nutriment that is net
			Feces	Urine	Methane				
		Cals.	*Per cent*	*Per cent*	*Per cent*		*Per cent*	*Per cent*	
Corn silage	237	4,606	31.7	3.6	8.5	68.3	10.7	45.5	66.7
Soy-bean hay	237	4,524	39.9	6.4	7.5	60.1	12.9	33.3	....
Do.	237	4,555	38.8	5.8	7.6	61.2	10.7	37.1	....
Average, soy-bean hay	237	4,540	39.4	6.1	7.6	60.6	11.8	35.2	58.1
Alfalfa hay	237	4,522	47.4	5.6	5.7	52.6	13.2	28.1	....
Do.	237	4,615	47.8	5.4	6.0	52.2	12.0	28.8	....
Do.	238	4,479	43.5	5.1	6.5	56.5	14.0	30.9	....
Do.	238	4,479	43.7	5.1	6.4	56.3	13.6	31.1	....
Average, alfalfa hay	238	4,524	45.6	5.3	6.2	54.4	13.2	29.7	54.6
Oats, ground	237	4,752	28.8	3.8	10.1	71.2	10.6	46.8	....
Do.	237	4,713	22.9	4.4	9.8	77.1	9.3	52.5	....
Average, oats, ground	237	4,733	25.9	4.1	10.0	74.1	10.0	49.7	67.1
Corn meal	238	4,558	7.9	3.9	10.4	92.1	9.5	68.3	....
Do.	238	4,558	8.8	4.3	11.0	91.2	8.8	67.1	....
Average, corn meal	238	4,558	8.4	4.1	10.7	91.6	9.2	67.7	73.9

It was the expectation of the authors, at a comparatively recent date, to determine net-energy values of individual feeds for body increase from such values for maintenance by multiplying the latter by a previously established ratio of net energy for body increase to net energy for maintenance; but it is now apparent that such a ratio obtained with a mixed ration would not correctly apply to its individual components (1) because, as shown in Table 24, the heat-increment values of different feeding stuffs are not proportional to their metabolizable energy values, and (2) because, as shown in

Figure 4, the metabolizable-energy value and the total heat-increment value, from which the net energy is computed, are not affected alike by the plane of nutrition.

Such a computation as above suggested would lead to the apparently unreasonable assumption that the effect of the plane of nutrition on the heat increment of the individual components of a ration is such as materially to alter their relation to each other. For example, in this experiment, whereas the heat-increment value for maintenance, of corn meal, has been found to be less than the heat-increment value of alfalfa hay, the heat-increment value for body increase, of corn meal, on the basis of the above assumption, would be greater than the corresponding value for alfalfa hay—as illustrated below, with data applying to the maintenance and the twice-maintenance periods, with steer No. 47.

Net-energy value of mixed ration for maintenance = 2,241 Cals.
Net-energy value of mixed ration for body increase = 1,557 Cals.
Ratio of net for body increase to net for maintenance = 0.695:1.
Heat-increment value of alfalfa hay for maintenance = 628 Cals.
Heat-increment value of corn meal for maintenance = 433 Cals.
Net-energy value of alfalfa hay for maintenance = 1,385 Cals.
Net-energy value of corn meal for maintenance = 3,111 Cals.
Net-energy value of alfalfa hay for body increase = 1,385 (net, maintenance) × 0.695 (factor) = 963 Cals.
Net-energy value of corn meal for body increase = 3,111 (net, maintenance) × 0.695 (factor) = 2,162 Cals.
Metabolizable-energy value of alfalfa hay for body increase = 2,013 Cals.
Metabolizable-energy value of corn meal for body increase = 3,544 Cals.
Heat-increment value of alfalfa hay for body increase = 2,013 (metabolizable) − 963 (net) = 1,050 Cals.
Heat-increment value of corn meal for body increase = 3,544 (metabolizable) − 2162 (net) = 1,382 Cals.

This demonstration of the fact that the metabolizable-energy values and the heat-increment values of the components of a ration, from which the net-energy values of these components are directly computed, are not affected alike by the plane of nutrition, leads naturally to the question of the causes of this situation.

These seem to be the effects (1) of the "difference method" of computation of the above-mentioned values of the components, and (2) of the fact, exhibited in Figure 1, that while the heat-increment and metabolizable-energy curves for the mixed ration are much alike, the heat-increment curve falls with decrease in the plane of nutrition, while the metabolizable-energy curve remains, in general, about on a level, these values of the ration as a whole, therefore, being differently affected by the plane of nutrition.

What change of basis of computation of these curves would tend to make them more nearly alike? Obviously, a lower value for the fasting metabolism—such, for instance, as the hypothetical value suggested on page 285. As has been stated, however, no satisfactory method for the derivation of such a value has been proposed.

The assumption, which is made the basis for computing the net-energy values of individual feeds for body increase in this paper (Table 22), is that the relation of the heat increments of the individual feeds to each other is not changed by the plane of nutrition; but that the magnitude of the heat increments of these individual feeds is affected in the same proportions as is the heat increment of the mixed ration composed of these feeds; and that the metabolizable-energy values of the individual feeds are also affected by the plane of nutrition in the same proportion as is the metabolizable energy of the mixed ration.

Two sets of such values were determined with each animal, representing the increase (1) from maintenance to half more than maintenance, and (2) from maintenance to twice the maintenance level of nutrition.

The heat-increment values used in computing these net-energy values of the individual feeds for body increase were derived from the corresponding heat-increment values for maintenance by increasing the latter in proportion as the heat-increment values of the mixed rations for body increase exceeded the corresponding values for maintenance; and the metabolizable-energy values of the single feeds for body increase were derived from the corresponding values for maintenance by decreasing the latter in proportion as the metabolizable-energy value of the mixed ration for body increase was less than the corresponding value for maintenance.

This new method of computation of net-energy values of individual feeds for body increase is not definitely and finally recommended, but is proposed for study, as one to which the authors have not yet found fatally disqualifying objections.

A question is raised as to the validity of such a computation by the character of the results obtained in these experiments. Thus, the ratio of the net-energy values of the mixed ration, and of the individual feeds for body increase (twice the maintenance) to the corresponding values for maintenance, would be as follows:

Steer No. 47, alfalfa hay,	634 : 1,385 = 0.465
No. 36, alfalfa hay,	605 : 1,395 = .434
No. 47, corn meal,	2,494 : 3,111 = .802
No. 36, corn meal,	2,477 : 3,059 = .810
No. 47, mixed ration,	1,557 : 2,241 = .695
No. 36, mixed ration,	1,531 : 2,218 = .690

The net-energy values (for body increase) of the corn meal are represented as being about four times as great as those of the alfalfa hay; and the ratio of the net-energy value of the corn meal for maintenance to the same for production is very much higher than is the ratio between the corresponding values for alfalfa hay. These values may be correct, aside from the errors incident to the use of the "difference method" involved in the computation of the net-energy value of the corn, but are surprising, and require confirmation.

The advantage of separating the total net energy of a ration into net energy for maintenance and net energy for body increase lies in the fact that by so doing is revealed the proportionality which exists between the different nutritive energy requirements of these activities, thus providing a convenient basis for computing the total feed requirement for a particular rate of body increase.

Table 23 is presented as an exhibit of the diversity of heat increments which would result from the comparison of the heat production at the different planes of nutrition, variously paired; that is, representing different parts of the curve of heat production.

This diversity of heat increments results from the fact that the heat production at various levels of nutrition is not a rectilinear function of the feed eaten. If the heat production were a rectilinear function of the feed, all these increments would be alike.

The character of these values emphasizes the necessity for the conventional standardization of the methods of determination of net-energy values.

A situation of general significance, applying not only to the results of the present study but to all determinations of net-energy value made by the methods followed at this institute, is that such values of individual feeds, either for maintenance or for body increase, but especially the latter, are not characterized by a consistency commensurate with the extreme particularity and refinement of the general method of experimentation.

This is a result of the fact previously observed (pp. 253 and 254) that the method of this determination depends in part only on physical and chemical measurements of great accuracy, but in part also on conventional assumptions and procedures which seem to be necessary for the derivation of simple and convenient measures of nutritive value, for guidance in practical feeding, but which assume a kind and degree of physiological orderliness which in reality does not exist.

The refined methods of work followed in this study are fully justified, however, by the fact that the objects of the experiments are not net-energy values alone, but embrace also the elucidation of principles of nutrition in general, for which purpose the most refined methods known fall short of ultimate if not of present requirements.

Another fact of general significance in this relation is that the whole method of experimentation in the determination of net-energy values of feeds presupposes that all essential nutrients other than those used for energy production are present in optimum quantities in all rations compared, so that nutritive energy values are unaffected by deficiencies of other than energy-producing nutrients.

It is the belief of the writers that this assumption, as relating to the energy studies of this institute, does not involve serious error, but it is their intention to undertake, at an early date, studies for the specific purpose of learning the magnitude of any such errors as may result from the suggested cause.

In regard to both of the above considerations it is important to understand that net-energy determinations may be of great value even though not absolutely accurate, since they are presumably much more nearly accurate than are the digestible nutrient values on which the most commonly used American feeding standards are based.

To illustrate certain facts in this relation the writers present in Table 24 an exhibit of a separation of the gross energy of several feeds into component fractions, as determined in recent experiments at this institute, leading finally, in the last column, to a statement of the percentage of the energy of the digestible nutriment of each feed which is net energy.

Obviously, if digestibility were of the same significance as a measure of energy value as is net energy (assuming that net energy is a true measure of energy value) the percentage of the energy of the digestible nutriment which is net would be the same, regardless of the nature of the feed.

These figures show, however, that this percentage varies from 54.6 for alfalfa hay to 73.9 for corn meal, the difference between these percentages representing the inaccuracy of digestible nutriment as a measure of energy value, and suggesting also that the most important advantage of net-energy values as compared with digestible nutrient values is that the former differentiate more nearly correctly than do the latter between the energy values of grains as compared with roughages; further, net energy serves to measure nutritive value in

terms of the product, thus indicating how much of the product will result from a given quantity of feed, while digestible nutrients are not characterized by any such significance.

SIGNIFICANCE OF THE RESULTS OF THIS STUDY IN RELATION TO PUBLISHED NUTRITIVE EVALUATIONS OF FEEDS

The relationship of the heat production to the plane of nutrition as revealed by this investigation, makes it impossible to assume, as was done in all papers from this institute which reported net-energy values of feeds, prior to and including a revision in 1925 (*10*) of the previously published net-energy determinations, that the efficiency of utilization of feed energy is the same above as it is below the maintenance level of nutrition.

The first papers in the light of the new understanding were one in 1926 (*9*), in which the different rate of utilization of the energy of feed for maintenance as compared with production of body increase was reported; and a second (*8*) in 1927, in which certain net-energy values of feeds for maintenance were published, though obviously such values are exactly true only at the point of energy equilibrium.

In all papers published from this institute prior to those above mentioned, which dealt with net-energy values, such diversity of heat increments as is exhibited in Table 23 was treated as an expression of experimental error—an assumption which has appeared less and less probably true from year to year, as the methods of experimentation involved in this work have been rigorously scrutinized and have been improved in many details. In the light of the results of the present study, therefore, such diverse heat increments as referred to might all be correct.

In so far as the net-energy values published by Armsby and Fries (*3*) and revised by Forbes and Kriss (*10*) are based on both submaintenance and supermaintenance feeding, they must be regarded, in the light of the present findings, as mixed values, but approximating more closely values for body increase than values for maintenance.

The net-energy values of feeds for cattle published by Armsby in the appendix of his book "The Nutrition of Farm Animals" (*2*) are based on the average composition and digestibility of American feeding stuffs, as compiled by Henry and Morrison (*14*), and on values for metabolizable energy and heat increment derived from the work of Armsby and Fries (*3*) and of Kellner and Köhler (*16*).

Armsby and Fries's experiments included both submaintenance and supermaintenance periods; while Kellner and Köhler's experiments (*2*, *16*, *17*), which contributed much to the derivation of the tables of values in Armsby's book, involve mostly supermaintenance periods.

Kellner and Köhler's starch values are, therefore, at least in the main, actual production values, and to this extent the significance of these values is unaltered by the present findings.

Armsby regarded the net-energy values in his above-mentioned book as applying both to maintenance and to body increase. From the nature of the computation by which these values were derived they must be regarded as representing more closely values for body increase than values for maintenance. In general it may be assumed that such of Armsby's values as are based on Kellner and Köhler's work represent production values, while values based on the experi-

ments of Armsby and Fries, especially values for roughages, have been affected by results of submaintenance feeding.

Møllgaard (*19*, *20*) has determined net-energy values of feeds by procedures in general harmony with the understanding of Kellner and of Armsby; and inasmuch as his basal rations were sufficient to cover the maintenance requirement of the experimental subjects, his determinations of net energy for production seem not to have been vitiated, as were some of Armsby's, by the use of data from submaintenance periods.

Møllgaard states energy values, for any nutritive purpose, in terms of net energy for increase of body substance. This procedure may serve, temporarily, as a basis for the computation of feed requirements, for practical purposes, but, in the light of the results of the present study, is imperfect in that it implies a constant relation between net-energy values of a feed for the different functions of maintenance, body increase, and milk production, irrespective of the kind of feed (see p. 293).

SUMMARY

A series of experiments was conducted with steers as subjects, both by the methods of direct and indirect respiration calorimetry, for the study of the energy metabolism in relation to the plane of nutrition, and related problems.

Five planes of nutrition were studied with each of two steers. As indicated by the quantity of feed given, these planes were (1) fast, (2) half of the maintenance requirement, (3) maintenance (energy equilibrium), (4) half more than maintenance, and (5) twice the maintenance requirement.

In the feeding periods the rations were composed of corn meal and alfalfa hay, in equal weights of dry substance, except in one period, with each steer, in which the ration was alfalfa hay alone.

An experimental unit, except as modified in the fasting periods, normally consisted of a 28-day interval, embracing a 10-day preliminary period on the experimental feeding treatment to follow, and an 18-day digestion period, the last three days of the 18 also constituting a continuous, respiration-calorimetric experiment.

Among the new experimental procedures employed in this study were (1) the adoption of the area of the removed hide as the measure of the surface area of the animal, (2) the use of the respiratory quotient, and (3) of the amount of the feed residues in the alimentary tract, as well as the heat production, as usual, as criteria in the standardization of the conditions for the determination of the fasting katabolism as the measure of the maintenance quota of net energy, (4) the correction of the heat production to correspond to a uniform live-weight and maintenance requirement of net energy, in the comparison of the heat production at different planes of nutrition, (5) a new method of computing net-energy values of individual feeds for body increase, based on the assumption that the heat-increment and metabolizable-energy values of the components of a ration are affected by the plane of nutrition in the same ways and degrees as are the heat increment and metabolizable energy of the ration as a whole, and (6) the use of a new procedure in computing the heat production to the standard day as to standing and lying.

The subjects were two Aberdeen-Angus steers, between 25 and 33 months of age, and throughout the study the results with the two steers agreed phenomenally well.

The heat production increased but little from fast to the plane of half of the maintenance requirement, and increased much more rapidly from the latter plane of nutrition to the maintenance level; but the entire rise in metabolism from the fasting to the maintenance level was at a distinctly lower rate than was the fairly uniform increase from the level of maintenance to that of twice the maintenance requirement.

The relation between the heat production and the food consumption, above maintenance, therefore, is expressed by a line of slight curvature and, below maintenance, by a pronounced curve.

The several factors which determined the curve of heat production in relation to the plane of nutrition seem to be (1) the amount of the observed heat production of fast (the base value), (2) the energy cost of utilization of food, (3) the difference in the energy cost of utilization of food for body increase as compared with the utilization of the same for the prevention of body loss, especially as affected by the specific dynamic effect of protein, by the "metabolism of plethora" (of fat and carbohydrate), and by the energy cost of synthesis of fat, and (4) the influence of the incompleteness of digestibility and metabolizability of the food on the proportions of the same which actually contribute to the heat production.

Another point of view, not in harmony with the accepted idea of the observed heat production of fast as the measure of the maintenance requirement of net energy, but depending on the hypothesis of a lower value for this datum, differing from the above by the amount of an assumed specific dynamic effect, or energy expense of utilization, of body nutrients oxidized during fast, would go far toward affording an explanation of this curve. Thus, in accord with this principle, this curve would be influenced by the dynamic effects of the differing proportions of food nutrients to body nutrients, and by the differing proportions of carbohydrate, fat, and protein of body nutrients, katabolized at planes of nutrition between fast and maintenance.

The curves of distribution of feed energy per kilogram of dry matter between feces, urine, methane, and heat increment, and digestible, metabolizable, and net energy, as affected by the plane of nutrition, were plotted.

From the plane of half of the maintenance to that of twice the maintenance requirement these curves were characterized as follows:

Digestible energy first rose slightly, on account of increased digestion of crude fiber, and then decreased, at an increasing rate, as a result of lowered digestion of carbohydrate and protein.

The curve of energy of the feces, naturally, was the reverse of that of digestible energy.

The curves of methane and urine energy were nearly straight lines, falling slightly with rise in plane of nutrition.

The curve of total heat increment (computed with reference to the fasting katabolism) rose at each period of observation, but each time at a decreased rate of rise.

The curve of total net energy (for maintenance and body increase together) was a practically straight line, falling rapidly with rise in

The curve of total heat increment was separated into components for maintenance and for body increase, that for body increase (computed with reference to the heat production at maintenance) being much higher than that for maintenance (computed with reference to the fasting katabolism).

From these heat increments and the corresponding metabolizable-energy values different net-energy values of the feeds for maintenance and for body increase were computed.

To such extent as the earlier net-energy values determined at this institute were affected by being derived in part each from the results of submaintenance feeding periods they are of mixed significance.

The net-energy values of the feeds for maintenance, with the two steers, were, for alfalfa hay, 1,385 and 1,395 Calories, for corn meal, 3,111 and 3,059 Calories, and for the mixed ration, 2,241 and 2,218 Calories, per kilo dry matter.

The corresponding net-energy values for body increase, at the plane of twice the maintenance requirement, were decidedly lower than the above, being for alfalfa hay, 634 and 605 Calories, for corn meal, 2,494 and 2,477 Calories, and for the mixed ration, 1,557 and 1,531 Calories. These net-energy values of feeds for body increase apply only to that portion of the total fed which is in excess of the maintenance requirement.

The corresponding net-energy values at the plane of half more than maintenance were in either close or exact agreement with those at the higber plane of feeding for body increase.

The proportion of the gross energy eliminated as methane varied from 6.42 to 9.83 per cent, the corresponding proportions of heat of fermentation (computed according to Krogh, but with a question as to the method) being 1.50 and 2.30 per cent, respectively. The sum, that is, the proportion of the gross energy, lost as methane and heat of fermentation together were 7.92 and 12.13 per cent, respectively. The lowest value of each of the above categories was found in the periods in which hay alone was fed, and the highest values in the periods in which the feed was half of the maintenance requirement of the mixed ration of hay and grain.

From this study it appears that comparable determinations of the specific dynamic effects of feeds, foods, or nutrients, can be determined only at the same plane of nutrition.

An important unsolved problem in the determination of net-energy value of feeds is the interrelated or reciprocal effect of the components of a ration on their respective energy costs of utilization, especially as affected by the plane of nutrition, since it is not known that the heat-increment value of a ration is, at all planes of nutrition, the sum of the heat-increment values of its components.

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STUDIES ON THE ETIOLOGY OF APPLE CROWN GALL[1]

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INTRODUCTION

The early history of crown gall on various hosts has been reviewed in detail by Hedgcock (*2*)[2] and by Smith, Brown, and Townsend (*11*). The stimulus imparted from their findings and the importance of this disease in California led C. O. Smith (*10*) to inoculate a number of hosts with the crown-gall organism (*Bacterium tumefaciens* Smith and Townsend) and thus to add further evidence of the cause and infectiousness of crown gall.

For almost a decade after Smith's publication (*11*), the hypothesis that practically all the various malformations that occur on root-grafted apple trees in the nursery were the result of infection by the crown-gall organism was generally accepted by plant pathologists. There were, it is true, a number of so-called doubtful cases, the etiology of which was not clear, but Riker and Keitt (*8*) in 1925 were the first to question seriously the current beliefs concerning the etiology of certain types of overgrowths found at graft unions and previously considered typical crown-gall formations. The experiments of Smith and his associates (*11*) on malformations of root-grafted apple trees were necessarily quite limited, and, as noted by Riker and Keitt (*9*) and later by Melhus (*3*), were not extensive enough to permit the drawing of definite conclusions.

Hedgcock (*2*) made an excellent classification, based on external characters, of the types of malformations on root-grafted apple trees. The type designated by him as woolly knot is the one studied chiefly by the writer. The woolly-knot type of malformation, which is the one most commonly found in the root-grafted apple nursery, is recognized by nurserymen and pathologists as numerically the most important. As shown by their illustrations, Riker and Keitt (*9*) were referring to this type when in 1925 they first reported their inability to obtain the crown-gall organism from a number of nursery trees rejected as crown-gall trees and when, somewhat later, they reported in detail their experiments and reiterated their previous findings, namely, that many types of malformation ordinarily diagnosed as crown gall were not due to infection by *Bacterium tumefaciens*, but were, in fact, wound overgrowths. Muncie (*4*) arrived at the same conclusion as the result of experiments performed along similar lines.

ISOLATIONS, CULTURES, AND INOCULATIONS

Smith and his associates (*11*) reported the isolation of an organism from what they termed the "hard gall" of apple, but it is not known definitely whether this was the woolly-knot or the hard-gall type.

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[1] Received for publication June 20, 1928; issued October, 1928. Credit is gratefully extended to R. B. Piper for most of the cultural and inoculation work in these experiments.

[2] Reference is made by number (italic) to "Literature cited," p. 313.

They classified the organism that they obtained as *Bacterium tumefaciens*, although it differed in cultural reactions and in reactions when inoculated on various hosts from the organism isolated from peach, daisy, and other hosts. They regarded the various forms as belonging to one species and described the organism isolated from the daisy as the type of the species. Attention is directed to the fact that the organism obtained from the apple by Smith et al. (*11*) failed to cause a reaction on the tomato inoculated with it. However, this organism infected the Paris daisy (*Chrysanthemum frutescens*). The reactions were not so pronounced as those obtained on this host by inoculations with the organism isolated from the peach.

That Smith and his coworkers recognized the fact that these organisms, obtained from different hosts and regarded by them as *Bacterium tumefaciens*, failed to cause infection when cross inoculated is evident, for under the caption "Quick tests for differential purposes" (*11, p. 115*), they recommend "inoculations into young, rapidly growing daisy shoots or into growing sugar-beet roots." It should be noted that the tomato, an easily grown host, was not recommended. These authors also continually refer to strains of *Bact. tumefaciens*, designating the daisy strain, the hop strain, and so on.

Riker and Keitt (*9*) and Muncie (*4*) noted the presence, in malformations on apple roots, of organisms that resemble *Bacterium tumefaciens*, but apparently all of their tests to determine the identity of organisms isolated from what they term "wound overgrowths" or malformations of any type were made on tomato, tobacco, or geranium, and not on Paris daisy, sugar beet, or Bryophyllum.

In the experiments reported here the daisy, as well as the tomato and other plants, has been used as a host to determine the identity of organisms isolated from malformations on apple-root grafts. For isolation purposes typical woolly-knot crown galls on the apple were selected. (Fig. 1, A, B, C, D.) This type of gall or malformation has almost invariably yielded an organism that appears to be identical with that described by Smith et al. (*11*) as the apple strain of *Bacterium tumefaciens* in its ability to cause reactions when inoculated into certain hosts. This so-called apple strain has been isolated from 38 of 52 galls similar to those illustrated in Figure 1. From the successful isolations, inoculations have been made into the daisy in every case and positive reactions have been obtained consistently. The types of reactions obtained on daisy and the controls are shown in Figure 2, A, B, C, and D. They generally consist of small intumescences ranging from one-eighth to one-quarter inch in diameter. Inoculations were made at intervals along the stem by means of single needle punctures from beef-agar cultures and the control punctures were generally made on the opposite side of the same shoot. In a number of cases the organism has been recovered from these galls and reinoculated into the daisy, resulting in infections.

A limited number of inoculations by means of single needle punctures from beef-agar slants have been made on small apple seedlings growing in the greenhouse. The seedlings were grown from open-pollinated fruit of the Chenango variety. Infections have been secured consistently with the apple organism, but none with the peach organism. Smith et al. (*11*), however, reported infection with the peach strain on apple. The type of infection secured on the apple in

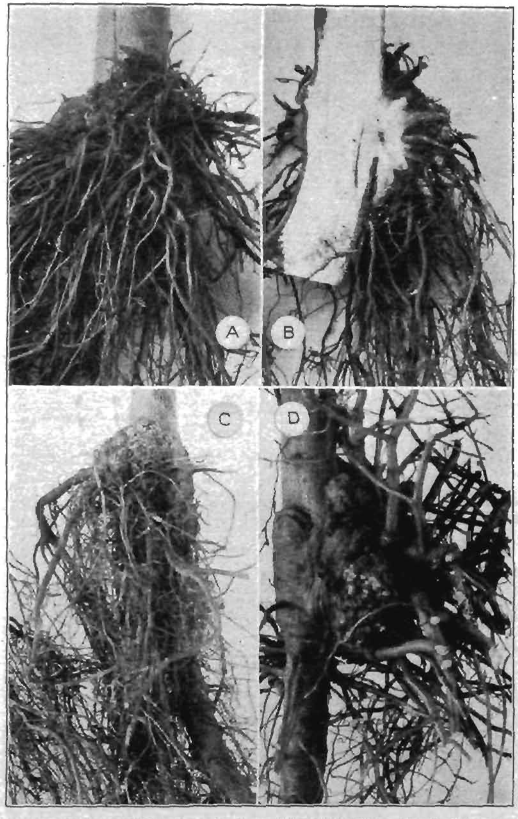

FIG. 1.—A, woolly-knot type of crown gall on 1-year-old Jonathan apple; B, longitudinal section of gall shown in A; C, woolly-knot type of crown gall on 1-year-old Stayman Winesap apple; D, woolly-knot type of crown gall on 1-year-old Wolf River apple. All natural size

FIG. 2.—A, reactions obtained on daisy with the apple-strain organism by means of single needle punctures; time, 60 days. B, control punctures made on the opposite side of stem in A; time, 60 days. *C, reactions obtained on daisy with the apple organism, isolated from gall shown in* Fig. 1, D, by means of single needle punctures; time, 60 days. D, control punctures made on the opposite side of stem in C; time, 60 days. All natural size

the writer's experiments consists of a protruding mass of root primordia, morphologically identical with what is considered typical aerial gall. (Fig. 3.)

The organism has been recovered from the specimen illustrated in Figure 3 and reinoculated into daisy, resulting in infections. Invariably the control punctures have healed over. Brown (*1*) reported negative results from attempts at isolations from aerial galls from bearing trees. The galls from which successful reisolations were made in the writer's experiment were very young, that is, 45 days from date of inoculation, and were apparently in better condition to yield a pathogene. The type of gall produced by Smith et al. (*11*), and illustrated in their Plate 12, 2, and by Brown (*1*) in her Plate 1, B, is in marked contrast with the aerial type of gall produced in the present experiments. From the report of Taubenhaus's experiments[3] it is possible that he may have isolated *Bacterium tumefaciens* from aerial galls on apple, but, unfortunately, he does not report having checked his isolations by means of inoculations.

A limited number of sugar beets have been inoculated with the apple strain and infections have been secured in approximately 50 per cent of the trials. Young sugar-beet seedlings were grown with 1 inch of the crown exposed above the soil. The exposed portion of the root immediately below the crown was washed with mercuric chloride solution, then rinsed with sterile water, and the organism introduced by means of one needle puncture at five different places. The control needle punctures were made in similar positions on the opposite side of the root. Figure 4, A, shows the small, fleshy root-like malformations produced on sugar beet by means of inoculations with the organism isolated from the apple gall illustrated in Figure 1, A. The control punctures are shown in Figure 4, B. In no cases have the controls shown the fleshy root formations illustrated here, although this type of root formation does appear infrequently on uninoculated roots, possibly through natural causes or natural infection. The reaction obtained is quite distinct from that resulting from inoculations with strains of crown gall obtained from hosts other than the apple. For comparison, attention is directed to Figure 4, C, which illustrates inoculation with a strain of *Bacterium tumefaciens* obtained from a peach gall. The apple-strain organism has been reisolated from the malformations produced on the sugar beet.

Inoculations made on the air plant *Bryophyllum calycinum* (Salisb.) with the apple organism have invariably resulted in malformations consisting of smooth, raised areas, approximately one-quarter inch in diameter. (Fig. 5, B.) The control punctures are shown in Figure 5, C. The reactions obtained on Bryophyllum by inoculations with the apple organism are more pronounced than those obtained on the daisy by inoculations with the same organism. They are not so pronounced as those obtained by inoculations with the peach organism. (Fig. 5, A.) In all instances, however, the inoculations with the apple-strain organism have been positive—that is, every needle puncture with the organism has resulted in infection—whereas none of the control punctures, made always on the opposite side of

[3] ADAMS, J. F. DISEASES OF FRUIT AND NUT CROPS IN THE UNITED STATES IN 1922. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul. Sup. 28: 308, 1923. [Mimeographed.]

FIG. 3.—Reactions obtained on an apple seedling, grown from a seed of an open-pollinated Chenango fruit, by means of single needle punctures with the apple organism isolated from the gall shown in Fig. 1, C. These reactions are morphologically identical with the so-called aerial galls. Time, 45 days. Natural size

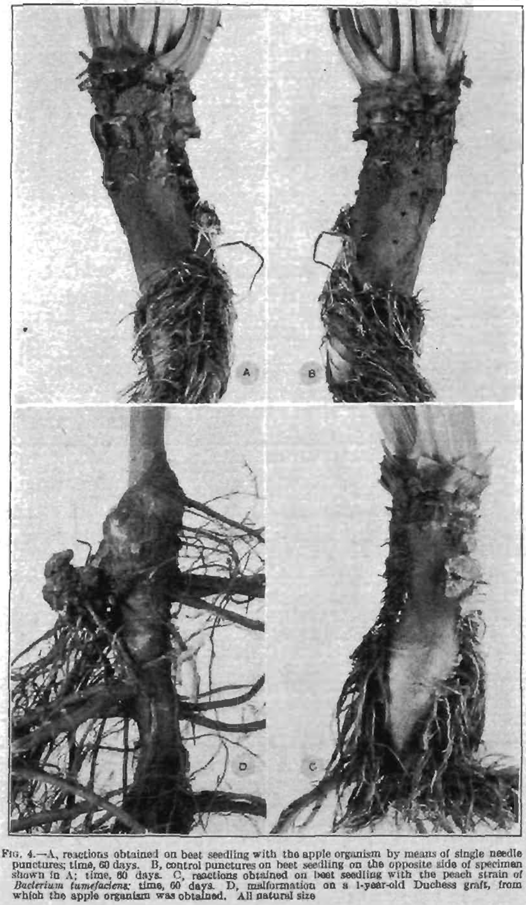

FIG. 4.—A, reactions obtained on beet seedling with the apple organism by means of single needle punctures; time, 60 days. B, control punctures on beet seedling on the opposite side of specimen shown in A; time, 60 days. C, reactions obtained on beet seedling with the peach strain of *Bacterium tumefaciens*; time, 60 days. D, malformation on a 1-year-old Duchess graft, from which the apple organism was obtained. All natural size

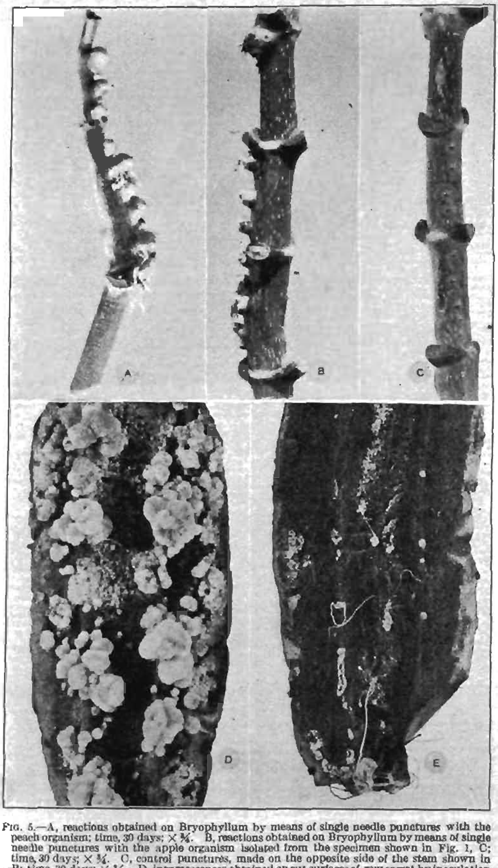

FIG. 5.—A, reactions obtained on Bryophyllum by means of single needle punctures with the peach organism; time, 30 days; $\times \frac{3}{4}$. B, reactions obtained on Bryophyllum by means of single needle punctures with the apple organism isolated from the specimen shown in Fig. 1, C; time, 30 days; $\times \frac{3}{4}$. C, control punctures, made on the opposite side of the stem shown in B; time, 30 days; $\times \frac{3}{4}$. D, intumescences obtained on cut surfaces of raw carrot by inoculation with the peach strain of *Bacterium tumefaciens;* time, 35 days; $\times 1\frac{1}{3}$. E, intumescences obtained on cut surface of raw carrot by inoculation with the apple organism; the areas appearing "frosty" were caused by normal cell proliferation; time, 35 days; $\times 1\frac{1}{4}$

the stem, has caused the slightest reaction. The organism has been recovered at will from the malformations produced on Bryophyllum by artificial inoculations, and plates poured from these areas have been practically pure.

Smith et al. (*11*) illustrated the intumesceuces obtained by inoculating cut raw surfaces of turnip with the daisy organism. Through correspondence the writer was informed that C. Stapp, of Germany, inoculates the cut surfaces of raw carrots in Petri dishes as a convenient method of testing the crown-gall organism. The writer, following this method, has made inoculations on carrots and has secured large intumescences on this tissue by inoculations with the peach strain (fig. 5, D), but has secured only slight intumescences approximately one-eighth inch in diameter by inoculations with the apple strain (fig. 5, E).

Inoculations into tomato, tobacco, geranium, and coleus with the apple-strain organism have been considered negative, although inoculation with the strain of *Bacterium tumefaciens* isolated from the peach always results in positive reactions on these hosts. The number of inoculations with the apple organism on geranium and coleus, however, has been too limited to permit the drawing of definite conclusions. Although inoculations on the tomato with the apple organism are considered negative, this statement should be qualified, since slightly water-soaked areas, absent from the control punctures (fig. 6, C), appear about the points of inoculation (fig. 6, B), followed by a slight disturbance of the tissues, as is shown in Figure 6, E. This plant was photographed approximately five months after the date of inoculation and the organism was recovered in practically pure culture.

Inoculations on the tomato and on the daisy with the peach organism are illustrated in Figure 6, A and D, respectively. A comparison between Figure 2, A and C, and Figure 6, D, is invited.

Since the results of inoculations depend so greatly upon the condition of the host, the inoculations made in these experiments are not tabulated. The general practice has been to make at least 10 needle punctures on the daisy stem with organisms isolated from the apple. Control punctures were made in the same manner. If only one of the inoculations resulted in infection the organism was considered the apple strain. Usually about 5 of the 10 points of inoculation resulted in infections. Whenever there was any doubt about an isolation it was subjected to repeated tests so that the result of hundreds of inoculations leaves no doubt about the ability of the apple organism isolated from the 38 galls of the woolly-knot type to perform as indicated here.

STRAINS OF THE CROWN-GALL ORGANISM

It has been deemed advisable to follow the suggestion of Smith et al. (*11*) and to call the organism obtained so consistently from the woolly-knot type of crown gall on apples the apple strain of *Bacterium tumefaciens*. These workers recognized and discussed in detail strains of the crown-gall organism and, although they concluded that "the ease with which . . . cross-inoculations take place points rather to one collective species," they also stated that "the differences [in the

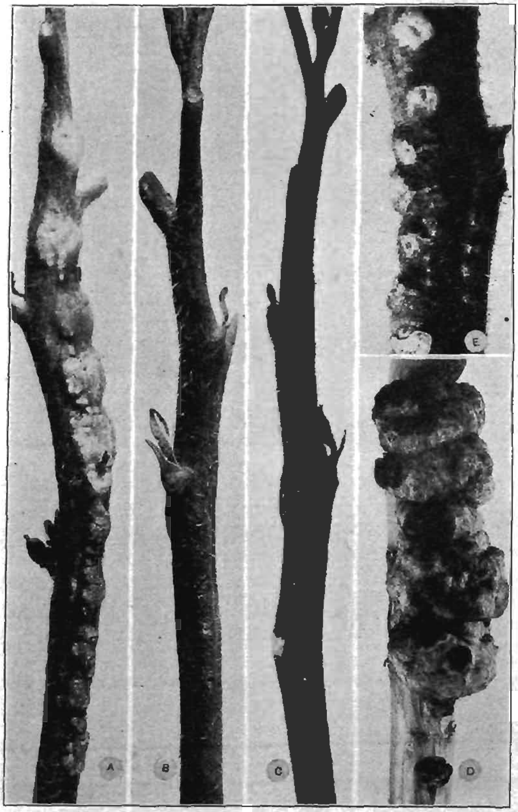

FIG. 6.—A, reaction obtained on tomato by means of single needle punctures with the peach strain of *Bacterium tumefaciens*; time, 30 days; × 1¾. B, slightly water-soaked areas (barely discernible in this illustration) at points of inoculation of the tomato with the apple organism; time, 30 days; × 1¾. C, control punctures on tomato; time, 30 days; × 1¾. D, typical malformations obtained on daisy by inoculations with the peach strain of *B. tumefaciens*; time, 90 days; × 1¾. E, slight disturbances on tomato at points of inoculation with the apple organism; time, 135 days; × 2

strains] . . . seem to be real differences, . . . [and] we do not know . . . what weight to give them as differential characters."

The conception that these several strains belong to one widely variable species is in conformity with the general conception of bacterial organisms. For example, the root-nodule organism (*Bacillus radicicola*) is generally accepted as consisting of several different strains, either partly or not at all cross infectious.

While it is probable that the organism repeatedly isolated from the apple in these experiments is identical with the one isolated from it by Smith et al. (*11*), this belief may be unwarranted, as they (*11, p. 100*) evidently believed that the culture they used for the determination of cultural characters had become contaminated. This is rather confusing since the organism used in these experiments agrees in cultural reactions with their apple organism. The fact that the cross inoculability of the two organisms agrees strengthens the belief that the two are identical, but the doubt as to their identity can not be dispelled in the absence of a comparison of the two organisms.

Patel (*5, 6*) has reported on the isolation of 15 strains of *Bacterium tumefaciens*. The organism isolated by Riker and Keitt (*9*) and by Muncie (*4*) from a type of gall occurring infrequently in the nursery, apparently is identical with the daisy organism of Smith et al. (*11*), if the ability to infect not only the daisy but also the tomato, the tobacco, and the geranium is accepted as a criterion.

That the reaction on culture media apparently is not always a reliable test for the crown-gall organism was noted by Riker (*7*). The organism isolated by him from the raspberry turned litmus milk pink, whereas the daisy organism, as had been noted by Smith and others, never did so. Riker (*7*), referring to certain morphological differences and cultural reactions, also calls attention to the fact that Smith had noted variations "as great as these between different strains of crowngall bacteria."

For the present, despite any morphological or physiological differences, it is deemed advisable to consider the organism used in these experiments as the apple strain of *Bacterium tumefaciens*.

DISCUSSION

Knowledge of the etiology of certain types of malformations found in root-grafted apple trees can not be fully rounded out until apple grafts can be grown in bacteriologically sterile soil.

The practical difficulties encountered in growing apple grafts on an extensive scale under aseptic conditions are well recognized. Riker and Keitt (*9*) and Muncie (*4*) used extreme care in an attempt to obtain aseptic conditions under which to grow apple grafts. Riker and Keitt (*9*), in a cultural examination of some of the trees resulting from these grafts, reported that "all were negative for the crown gall organism," but stated that some of the grafts examined were not sterile. Muncie (*4*), in an experiment conducted similarly, reported the absence of *Bacterium tumefaciens* but at the same time noted the presence of bacteria in the galls examined, and called attention to the fact that the plates "were flooded . . . and [then the suspension] inoculated into tomato [plants]" with negative results. Here again the tomato and not the daisy was used to test the organisms that were isolated. Thus it is evident that Riker and Keitt (*9*) and

Muncie (*4*) were unable to obtain sterile conditions for growing apple grafts and that their isolations from so-called wound overgrowths were tested on hosts that are not susceptible to the organism isolated by the writer. But regardless of these facts it should be noted that Riker and Keitt's Plate XLV, E, (*9*) and Muncie's Plate III, B, (*4*) do not represent the types of malformations discussed in this paper. In other words, they are not the woolly-knot type of crown gall. They are considered not to be the types of malformations commonly encountered in the nursery and greatly resemble the specimen illustrated in Figure 4, D, which is a Duchess apple tree grown from a graft with a very poor union. While the apple strain of the crown-gall organism actually was isolated from this specimen, it is still possible that callus formation of nonpathogenic origin may have played the more important rôle in causing this malformation. Patel (*6*) and others have isolated the crown-gall organism from the soil and it is conceivable that the organism might be recovered quite readily from apparently healthy graft unions, just as the pear-blight organism (*Bacillus amylovorus*) often may be found streaming over healthy bark.

Just how important a rôle this apple strain of *Bacterium tumefaciens*, if in fact it is a strain of that organism, plays in the formation of the woolly-knot type of malformation is still undetermined. Evidence is produced showing that it is quite constantly associated with these malformations; that it is pathogenic to a limited degree on daisy and apple shoots, as well as on sugar beet, and causes malformations on them, and that it produces even more pronounced malformations on Bryophyllum. It will produce, however, only a barely perceptible reaction on tomato and tobacco. It has also failed, in limited tests to cause a reaction on geranium. These hosts were used by Riker and Keitt (*9*) and by Muncie (*4*) for testing organisms that they isolated from malformations designated by them as wound overgrowths. Most of these overgrowths apparently are identical with the type of crown gall known as woolly knot and used in these experiments.

SUMMARY

Smith, Brown, and Townsend isolated a bacterial organism from malformations on root-grafted apple trees. This organism, which was designated as the apple strain of *Bacterium tumefaciens* Smith and Townsend, caused local reactions when inoculated into the Paris daisy, but no reaction when inoculated into the tomato.

Isolations have been made from types of malformations on grafted apple trees, known as woolly-knot crown gall. Isolations from these types of malformations have consistently yielded an organism that appears to be identical with the organism which Smith et al. designated the apple strain of *Bacterium tumefaciens*.

When Paris daisy, apple shoots, sugar beet, and Bryophyllum are inoculated with the apple organism, definite and pronounced galls or malformations are produced, whereas when tomato and tobacco are inoculated with this organism no definite galls are produced, although extremely slight distrubances of the tissues occur on these hosts. These results demonstrate the necessity of using the proper host, in receptive condition, in testing the infectiousness of organisms isolated from malformations on the roots of apple trees.

When apple shoots are inoculated with the apple organism malformations morphologically identical with the so-called aerial crown gall are produced. The organism has been reisolated from these artificially produced aerial galls.

The degree of pathogenicity that this apple strain of *Bacterium tumefaciens* exhibits on apple shoots and on host other than the apple does not necessarily prove its rôle as a pathogene in connection with malformations on the roots of grafted apple trees; nor does its constant association with these malformations furnish conclusive proof that it is the causal agent. These facts, however, should be included in any consideration concerning the nature or cause of these malformations.

While emphasis is placed on the desirability of growing apple grafts in bacteriologically sterile soil before passing full judgment on the question of the etiology of these malformations, the facts given here are considered to support the hypothesis that the apple strain of *Bacterium tumefaciens* can cause certain types of malformations or galls which occur on root-grafted apple trees.

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